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Translation

ANTHRAX

Ed. by

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ANNOTATION

This book describes one of the most dangerous diseases of animals and man. Brief information from the history of the disease and its occurrence in our country and in other countries of the world is presented. The pathogen, the disease symptoms, and the pathoanatomical changes observed in sick animals are described.

Much attention is devoted to the problems associated with treating sick animals, immunity, and veterinary and sanitary measures implemented at farms when anthrax appears.

INTRODUCTION

Anthrax is one of the dangerous diseases with an acute course. In the past it inflicted tremendous losses upon animal husbandry, and it often evoked mass illness among people.

There were no effective ways to control this disease, and it was only after the pathogen was discovered that intensive work began on questions of the microbiology, epizootiology, and diagnosis of anthrax. Scientists answered them successfully at the end of the past century and in the first half of the present century.

Especially great changes in the methods for controlling infection occurred after acquisition of anthrax vaccines, owing to which a possibility arose for preventing the disease. The vaccines were proposed by L. Pasteur (1881) and L. S. Tsenkovskiy (1883). Immunity was studied and disinfection methods to be used with anthrax were developed in parallel.

As of today the questions noted above have been studied sufficiently well, but before we can provide fully conclusive answers to them, we must do further work to reveal, record, and eliminate infection foci in the soil. Mass animal vaccination alone would not insure total elimination of the disease, since the threat of arisal of anthrax among unvaccinated animals would constantly arise.

Despite the complexity of the measures being implemented to control anthrax, its epizootics have been eliminated from our country. The present problem is to eliminate known anthrax foci and discover those yet unrevealed.

Scientific research and practical efforts of the last 15 years showed that the great and difficult task of eradicating anthrax--a dangerous infection of the community of man and animals, may be completed successfully through joint implementation of measures by veterinary and medical services.

The main ways to eliminate anthrax are to vaccinate animals in areas harboring this disease, reveal and study infection foci in the soil and decontaminate them, and study the ways of the pathogen's spread, as well as a number of other problems.

This book describes anthrax control methods proposed by domestic and foreign scientists. In addition to known methods, it presents new ones being used in microbiology as well as in the diagnosis of the disease, in the treatment of patients, and in disinfection. Theoretical aspects having to do with microbiology, epizootiology, pathogenesis, immunity, and a number of other problems are illuminated as well.

The book is intended for scientists studying anthrax, and for practical workers employed by sovkhoz and kolkhoz veterinary services and diagnostic laboratories.

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HISTORICAL SURVEY

Anthrax has been known to mankind as an infectious disease of animals since ancient times. It was diagnosed in many countries of the world where the geographic conditions, soil, and temperature favored survival and development of the pathogen in the environment. The spread of anthrax in those distant times was promoted by development of states and nations, by migrations of people and animals over great distances, by growth in the strength of ties between states, and by wars. A disease with symptoms recalling anthrax is even described in the Bible. It was observed among animals as well as people as a carbuncular disease.

A disease having clinical signs recalling anthrax was also observed in Ancient Greece. Thus in the "Iliad" (Book 1) Homer describes a disease that arose in the Greek encampment during the seige of Troy in 1218 B.C.; it first infected mules and dogs, and then people. A similar disease is mentioned in the works of Hippocrates (460-377 B.C.). According to him the island of Aegina suffered very strongly from this disease in 1295 B.C., as did the Thessalian city of Kranok in 430 B.C.

Nor was anthrax a rare phenomenon in Ancient Rome. Many historians and poets of this country described it under various names. Plutarch wrote that violent outbreaks occurring during the time of Romulus killed animals and people very quickly. Dionysius of Halicarnassus and Titus Livius asserted that it wrecked cruel havoc among farm animals and caused the death of large numbers of people in 463, 453, and 452 B.C.. Titus Livius reported a disease which arose first among animals and then infected people in 433, 428, 400 and 175 B.C. Pliny the Elder (Gaius Secundus) mentioned a carbuncular epidemic in Italy in 163 B.C., and he believed that the disease had been imported there with woolen goods from the Narbonne district (Southern Gaul); Cato, Varro, and Lucretius called the disease sacred, or volatile, fire (ignis sacer). It was also described by Aulus Cornelius Celsus, who lived at the end of the 1st century B.C. and the beginning of the 1st century A.D. (S. F. Khotovitskiy, 1831).

In his poem "Georgics" (37-30 B.C.), the Roman poet Virgil accurately described a disease observed among animals of different species and man, leaving no doubt that the reference was to anthrax.

It is also mentioned in other ancient writings. There are several mentions of anthrax in the 10th century collection of veterinary writings entitled "Hippiatrica". In the opinion of some authors it is also described in the "Quadruped Medicine" attributed to the 11th century (D. M. Klemm, W. R. Klemm, 1959).

The first reports of anthrax on Russian territory may be found in the Nikonovskaya Chronicle (979 A.D.), which indicates that many domestic and wild animals as well as people died. The Tverskaya Chronicle (the entry for 1158) offers evidence of the death of horses, cattle, and people in Novgorod. An epizootic recurred in this city in 1204.

In 1284 epizootics caused the death of farm animals not only in Russia but also in Poland, Lithuania, and other neighboring countries. Many horses, cattle, and sheep fell. Anthrax was probably among the diseases causing these animal deaths.

V. M. Koropov presents interesting data from Russian chronicles in the book "Istoriya veterinarii v SSSR" [History of Veterinary Medicine in the USSR] (1954). Thus major epizootics were observed among animals of different species and epidemics occurred among people in 1309, 1374, 1375, 1443, and 1448. In 1640 the disease appeared among people, its origin being associated with removal of skins from deceased animals. The same thing happened again in 1643 in the vicinity of the city of Ostashkov.

In connection with the violent epizootic in 1640, a Czarist ukaze prohibited the removal of skins from sick and fallen animals, and required their carcasses to be buried deep in the ground. It was decreed that violators were to be whipped absolutely without mercy. Hence it follows that by this time, the infectiousness of the disease and the danger of human contact with the skins and hides of animals dying from or stricken with anthrax were well known in Russia.

By as early as the mid-1350's quarantines were introduced into the major trading cities of Italy as a means for isolating foreign vessels, people, and goods arriving in the country for the first time. Beginning with the 16th century quarantine measures began to be implemented in many countries of Europe, to include Russia. However, despite the steps taken, anthrax continued to spread, and to cause considerable losses. It is evident from descriptions written by Kirkh in 1658 that the disease broke out among cattle and then among people in 1617. About 60,000 persons died (S. M. Derizhanov, 1935).

There are indications that anthrax spread through Germany and France in 1712; through Poland, Silesia, and Saxony in 1726; through many European countries in 1756; through Finland in 1758 and 1774; through Sweden in 1774. According to a report by S. F. Khotovitskiy it caused the death of 85,000 horses in Siberia in 1781.

It is interesting that even in 1831, concluding his historic survey, S. F. Khotovitskiy wrote in the book "O sibirskoy yazve" [On Anthrax] that with time, the clinical signs and manifestations of "*ognevik*" (anthrax) underwent change: In ancient times it supposedly affected people more, in the Middle Ages it manifested itself in the form of leprosy, and in the present more animals fall ill with it, and people are rarely affected. That such notions are mistaken is obvious. Therefore in this historical survey we attempted to cite only those sources which provide descriptions of illness observed simultaneously in different species of animals and people, which is typical of anthrax.

But all these sources were nothing more than passing references to cases of disease, in which case the authors very often described carbuncular and other epizootics with the general terms ignis sacer, ignis volatilis, and ignis S. Antonii, treating

diseases such as human and cattle plague, anthrax, and rabies as all the same. Therefore many reports by authors writing in ancient times and in the Middle Ages should be treated very cautiously. It was not until the late 17th and early 18th centuries that the first scientific works appeared, in which the symptoms of the disease, its causes, and its occurrence were described, and recommendations on the treatment of sick animals were even offered. One of the first such works is that of Ramazzini, who described an epizootic that dominated almost all of Italy in 1690-1691. He was the first to distinguish carbuncular diseases from cattle plague, but he did not isolate anthrax as a separate nosological unit. This was first done by the Russian physicians Abram Eshke and Nikita Nozhevshchikov employed by the Kolyvan'-Voskressensk plants of Altayskiy Kray. They presented detailed information on anthrax to the medical board. In his work, A. Eshke (1758) described symptoms of the disease in people, he pointed out its similarity to disease in animals, and he noted its seasonality. In 1762 N. G. Nozhevshchikov made a detailed study of the symptoms of the disease and the conditions promoting its arisal.

Among foreign researchers, the first to offer a scientific description of anthrax was Moran in France. In 1766 he submitted a report on this disease to the Academy of Sciences in Paris (12).

In 1769 Fournier distinguished anthrax as a separate nosological unit.

Other works providing detailed descriptions of anthrax appeared as well. In the 1770's P. S. Pallas observed and described it in the Urals, along the Irtysh River, in the vicinity of Ishim, and in the Kirghiz steppes. In 1780 M. Tomassen received a decoration from the Dijon Academy for his research on this disease.

S. S. Andreyevskiy (1786-1789) contributed much labor to the study of anthrax. Following a 3-year expedition into the Chelyabinsk District of the Ural'sk Vice-regency, in 1788 he presented the medical board his essay "On Anthrax" containing a detailed map of the disease's occurrence, a description of the signs of the diseases, and illustrations providing an impression of the size and internal structure of carbuncles appearing on animals and man (9). In this same work the author described an experiment he performed upon himself in the presence of Doctor V. Zhukovskiy, Chelyabinsk Governor Shveygofen and Judge Olovyannikov, in which he demonstrated the infectiousness of the disease and its identity to that observed in animals and man.

By this experiment S. S. Andreyevskiy wanted to confirm the infectiousness of the disease and its identity to that observed in animals and man.

Moreover S. S. Andreyevskiy is credited with the main role in defining the disease, which he named Siberian ulcer after the place in which he studied it, and in describing the methods of the disease's prevention and treatment.

Many other researchers continued to study anthrax subsequently. In 1790 it was described by I. Peterson, and in 1792 M. L. Gamaleya published his book. V. Kozlov (1795), V. Zhukovskiy (1795), and Pinyayev (1797) submitted essays to the medical board in which they indicated a number of interesting ideas they had come up with. Thus M. L. Gamaleya reported the possibility for transmission of infection from sick to healthy animals by blood-sucking insects; V. Kozlov suggested the hypothesis that tiny invisible living beings were the cause of disease.

It was the 19th century. Anthrax epizootics continued to rage, and they were recorded in different countries almost every year. Especially large outbreaks were observed in West Europe in 1803, 1807, 1811, 1822, and 1834. According to estimates by (Delafon) more than 284,000 sheep were killed by this disease in 1843 just in the small province of (Bos') (in Orleans) (P. Lyubimov, 1867).

It is believed that anthrax was brought into the USA by the first settlers from Europe. Major outbreaks were noted there among domestic animals and people in 1835, 1851, and 1884.

In Russia, anthrax epizootics were registered several times in the period from 1807 to 1857. For example many deer, and even wolves feeding on carcasses, died in Lapland in 1823. The disease also spread among cattle, sheep, and people. Deaths among just domesticated deer alone exceeded 5,000 (13).

This situation alarmed veterinary specialists studying anthrax. Books were published by F. Uden (1807), Fr. Geyrot (1807), and Ellizen (1808). In 1823 Barthelemy managed to demonstrate the disease's contagiousness. S. F. Khotovitskiy's book "On Anthrax" was published in 1831, F. Shkinskiy's book "On Siberian Fire Disease" was published in 1832, and V. I. Vsevolodov's "Experience in Studying Epidemic Diseases Among Domestic Animals" was published in 1846. These books provide an exhaustive description of the disease.

In 1850 Davaine and Rayer found thin, cylindrical, motionless rods in the blood of animals stricken and killed by anthrax. In 1855 Pollender reported that back in 1849, when he autopsied cows that had been killed by this disease, he found their blood to contain "rod-shaped, very thin bodies, dense in appearance and completely straight and motionless."

Professor F. Brauel' of the Derptskiy (presently Tartu) Russian Veterinary School conducted research confirming the existence of these rod-shaped bodies, and he described them in 1857-1858. As a result of numerous blood innoculations he also managed to experimentally infect different animals. In 1863 Davaine proved the capability blood containing anthrax bacilli had for causing disease. He established that rod-shaped elements appear in the blood of sick rabbits 5 hours prior to death at the earliest, and that it is only from this moment that the blood acquires the capability for evoking illness in other animals.

R. Koch reported in 1876 that the formations under examination were microorganisms which reproduced by division and which were capable of developing on nutrient media. He also proved that such cultures could evoke anthrax in animals.

Louis Pasteur performed similar research in 1877. He obtained a pure culture of anthrax pathogen, studied its development in artificial nutrient media, and established that culture filtrate was harmless and that the infectious element could survive in soil for long periods of time. In 1881 he created anthrax vaccines 1 and 2, which was an outstanding achievement.

A culture of anthrax microbes was obtained in Russia for the first time by the prominent scientist V. K. Vysokovich in 1882, and in 1883 L. S. Tsenkovskiy prepared anthrax vaccines 1 and 2 and successfully completed sheep vaccination experiments, thus initiating brilliant research on prevention of anthrax among agricultural

animals by vaccination. Despite this, anthrax continued to arise both in Russia and in many countries of the world in the latter half of the 19th and start of the 20th century.

Nocard and Leclainche (19) reported significant spread of anthrax and considerable losses in animal husbandry due to it in France, Italy, Great Britain, Germany, Spain, Belgium, and elsewhere. They also cited data on the occurrence of anthrax in Australia and in American and Asian countries.

According to V. F. Nagorskiy 643,303 animals caught anthrax in 60 provinces of European Russia from 1885 to 1899; of these, 517,924 head died. During the same period 50,473 animals fell ill in 79 districts of Germany; of these, 46,898 head died.

According to N. A. Mikhin (1942) from 32,000 to 60,000 animals caught anthrax each year from 1900 to 1912, which is an annual average of 47,976 head. Sizable anthrax epizootics also broke out among reindeer in the country's north.

Anthrax was also highly prevalent in the country during the first years of Soviet rule, which elicited great concern. It was in this connection that the RFSFR Soviet of Peoples Commissars published a decree on 10 October 1927 ordering the RFSFR Peoples Commissariat of Agriculture to develop measures to control it.

A group of scientific and practical veterinary specialists was created in compliance with a directive of the RFSFR Peoples Commissariat of Agriculture. In 1928-1929 this group implemented a complex of measures against anthrax in the Belgorod District of the Central Chernozem Province. Their work was published in the journal PRAKTICHESKAYA VETERINARIYA, No 11-12, 1929. The obtained results were extensively employed in measures against anthrax throughout the entire country, which dramatically reduced the disease's occurrence. For example by 1950 enzootic and epizootic outbreaks of anthrax were completely eliminated.

Following rehabilitation of the war-devastated national economy, the principal measures for controlling anthrax included mass vaccination of animals and implementation of veterinary and sanitary measures. As a result in the last 26 years (from 1947 to 1973) the number of susceptible locations in the country decreased by a factor of 21.33, the number of animals falling ill dropped by a factor of 20.57, and the number of animals dying decreased by a factor of 20.1.

However, the possibility that sporadic cases of this infection may occur does exist. This can be explained by the biological features of the pathogen, by its ability to survive in soil for long periods of time, and by our still-inadequate knowledge of former centers of infection.

In some countries of Europe anthrax arises today only sporadically, and it apparently offers no major threat. However, in some countries it arises periodically in the form of epizootic outbreaks. According to data of the International Epizootic Bureau, in 1959-1964 7,871 anthrax outbreaks were registered in Greece, 1,837 were registered in Italy, and 1,215 were registered in Spain. In Great Britain, 1,215 outbreaks of animal anthrax were registered in 1952, and 609 were registered in 1953 (according to a report on the work of the veterinary service).

Anthrax outbreaks have also been registered in Asian countries--in Iran, Turkey, India, Syria, and elsewhere. Anthrax is also observed in American countries. Sporadic cases of anthrax arise yearly in the United States of America. Nevertheless data of the International Epizootic Bureau show that there has been a clear tendency for the disease to decline in the period from 1968 to 1972.

BIBLIOGRAPHY

1. Vsevolodov, V. I., "Opyt ucheniya o poval'nykh boleznyakh mezhdu domashnimi zhivotnymi" [Experience in Studying Epidemic Diseases Among Domestic Animals"], St. Petersburg, 1846.

2. Gamaleya, M. L., "O sibirskoy yazve i o yeye narodnom lechenii" [On Anthrax and on Its Folk Treatment], PERM', 1792.

3. Zemmer, Ye. M., VETERINARNYY VESTNIK, Khar'kov, No 3, 4, 5, 1882, p 105.

4. Zemmer, Ye. M., VETERINARNYY VESTNIK, Khar'kov, No 6, 1882, p 1.

5. Koropov, V. M., "Istoriya veterinarii v SSSR" [History of Veterinary Medicine in the USSR], Moscow, Sel'khozgiz, 1954.

6. Lebedev, N. D., PRAKTICHESKAYA VETERINARIYA, No 11-12, 1929, p 1962.

7. Liviy, Tit., "Rimskaya istoriya ot osnovaniya goroda" [The History of Rome Since the City's Founding], Moscow, 1899.

8. Nagorskiy, V. F., "AVN," Book 7 (Appendix), 1902.

9. Palkin, B. N., ZHMEI, Vol 7, 1951, p 81.

10. Peterson, I., "Kratkoye opisaniye bolezni v Rossii, nazyvayemoy vetryanoy i sibirskoy yazvoy" [A Brief Description of a Disease in Russia Called Wind and Siberian Ulcer], Tobol'sk, 1790.

11. Publiy Vergiliy Maron, "Sel'skiye poemy Bukoliki, Georgiki" [Pastoral Poems of the Bucolics and Georgics], Moscow, 1934.

12. Rozen'yer, L. A., "Sibirskaya yazva u cheloveka" [Anthrax in Man], Kishinev, 1948.

13. Khotovitskiy, S. F., "O sibirskoy yazve" [On Anthrax], St. Petersburg, 1831.

14. Tsion, R. A., PRAKTICHESKAYA VETERINARIYA, No 11-12, 1929, p 978.

15. Shkinskiy, F., "O sibirskom ognevike" [On Siberian Fever], St. Petersburg, 1832.

16. Brauell, F., "Archiv. f. path. Anat. und Phys.," 1858, p 4.

17. Davain, C. I., C.R. ACAD. SCI., Vol 57, No 4, 1863, p 220.

18. Koch, R., COHN'S BEITRAGE ZUR BIOLOGIE DER PFLANZEN, Vol 2, 1876, p 276.

19. Nocard, E., and Leclainche, E., "Mikrobnyye bolezni zhivotnykh" [Microbial Diseases of Animals], St. Petersburg, 1906.

20. Pasteur, L., Chamberland, Ch. Ed., and Roux, E., C.R. ACAD. SCI., Vol 92, 1881, p 429.

21. Pollender, F. A. A., VIERT. F. GERICHTL. U. OFF. MED. BERLIN, Vol 8, 1855, p 103.

BIOLOGY OF THE PATHOGEN

Anthrax bacillus was discovered microscopically for the first time by Pollender in 1849. In 1850 the French scientists Devain and Rayer discovered filamentous, motionless bodies in the blood of sheep stricken and killed by anthrax. The same sort of bodies were observed in Russia among dead animals by Professor F. Brauel' of the Derptskiy Veterinary School (1857). He was one of the first to discover bacilli in the blood of a person who died from anthrax, and to experimentally reproduce the typical disease in animals by infecting them with blood containing microscopically visible bacilli (vibrios).

However, the significance of these bacilli remain unclarified for a long period of time, and it was not until 1863 that Davain conclusively established the role of these formations as anthrax pathogens. Thus 1863 is said to be the official date of discovery of anthrax bacillus. The rod-shaped bodies described by this scholar were named anthrax bacteria (Bacteri des charbonneus).

(Delyafon) attempted to obtain a culture of the possible anthrax pathogen back in 1860, but it was not until 1876 that pure cultures of anthrax bacillus could be isolated, first by R. Koch and then by L. Pasteur. Using these cultures, they reproduced the disease in animals independently of one another.

Research by R. Koch in 1876 and by L. Pasteur in 1877 proved that vegetative cells of the anthrax microbe are capable of forming spores.

In 1888 Serafini discovered that anthrax bacilli formed capsules.

In Russia, an anthrax microbe culture was first obtained by V. K. Vysokovich in 1882. In subsequent years the biology of anthrax pathogen was studied by L. S. Tsenkovskiy, I. N. Lange, P. N. Andreyev, N. A. Pokshishevskiy, N. A. Mikhin, S. N. Vyshelesskiy, R. A. Tsion, M. V. Revo, Ya. Ye. Kolyakov, F. A. Terent'yev, and many other scientists.

The anthrax pathogen--*Bacillus anthracis* (F. Kon, 1872) is classifed as order Eubacteriales, family Bacillaceae, and genus and subgenus *Bacillus*. This genus contains about 25 aerobic and facultative-anaerobic bacteria reacting positively to catalase.

The following species are most closely associated with anthrax bacillus: *Bac. cereus* sive *Bac. anthracoides* sive *Bac. pseudoanthracis* (waxy bacillus), *Bac. cereus* var. *mycoides* sive *Bac. mycoides* (root-like bacillus), *Bac. megaterium* (cabbage bacillus), *Bac. subtilis* sive *Bac. mesentericus* Trevisan (grass bacillus, also referred to in

the literature as potato bacillus), and *Bac. pumilus* sive *Bac. mesentericus* Chester (the latter synonym is often used for potato bacillus).

They are all saprophytes with the exception of *Bac. cereus*, which synthesizes an active enzyme of pathogenicity, lecithinase, and which is capable of eliciting dietary toxicosis.

Bac. anthracis is a rather large (from 1-1.3 to 3-10 μ) Gram-positive, nonmotile rod forming capsules and spores. It is encountered in three forms: a vegetative form--cells of varying size (encapsulated and nonencapsulated), a spore form--spores encased within a well expressed exosporium, and a spore form in which the spores exist in isolation from one another.

Unstained bacterial cells in preparations made from the blood and tissues of animals stricken or killed by anthrax may have the form of homogeneous transparent rods with slightly rounded ends.

The rods lie separately, or they are joined together into short chains. The number of cells in a chain of highly virulent strains does not exceed three, while there may be more with strains exhibiting low residual virulence.

Lipoprotein granules, located subterminally and terminally for the most part, have been discovered in the bacteria.

The morphology of unstained bacteria from cultures grown in solid or liquid nutrient media is similar to that described above; however, in this case the cells always form more or less long chains.

Rods contained in smears from cultures of strains that exhibit typical growth as a flaky precipitate in liquid nutrient media arrange themselves in long chains (Figure 1). Bacteria from cultures exhibiting atypical diffuse growth in liquid media form short chains.

The cells of varying length recall cylinders with perfectly rounded margins. The surface of the cell wall is uneven.

In stained preparations, the ends of rods forming chains face one another; they are straight and closely clipped. Free ends, meanwhile, are slightly rounded. Sometimes the chains look like bamboo reeds, the clipped ends of the microbial cells are sometimes dented inward, and they are symmetrically thickened at their points of attachment. Such forms are encountered among bacteria synthesizing a capsule when growing in media containing proteins, or among bacteria reproducing in the animal body.

The bacterial cell has a nucleus. The first differentiated nucleoid (nuclear apparatus) was observed in anthrax bacillus by F. Ya. Kitayev (1922), who defined it as a chromidial apparatus. He noted concurrently that the nuclear element takes part in division of vegetative cells, and that it is seen in germinating spores.

Later the existence of a formed nucleus in anthrax bacillus was confirmed by Flewett (1948).

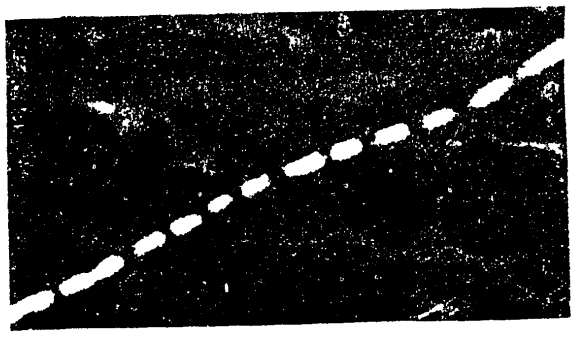

Figure 1. Vegetative Cells From Broth Culture: Luminescent microscopy. ML-2 microscope. MNF-10. Objective 95 x 1.25; ocular photo 012.5 x.

In 1952 M. N. Meysel' and L. V. Mirolyubova reported that the nucleus of vaccine anthrax strains has the form of helical strands occupying the central part of the cell and arranged along its axis.

According to Chatterjee and Williams (1962) the chromatin bodies of cells in young cultures were long, continuous formations located centrally. Both continuous and dividing nucleoids were present in mature cells. Long chromatin bodies in cells in 24-hour cultures arranged themselves as large complexes consisting of spherical formations.

Using cytochemical tests, the above authors concluded that RNA is associated with the cytoplasm of the bacterial cell while DNA is associated with the nucleoids, and that anthrax bacillus possesses a differentiated, discrete nucleoid.

G. V. Dunayev (1967, 1972) also discovered a differentiated nucleoid in vegetative cells from Tsenkovskiy and STI-1 No 2 vaccine strains fixed and stained by the classical Pekarskiy-Robinou method.

Vegetative anthrax bacillus cells contain a clearly outlined nucleoid in all phases of development of the bacterial culture.

The nuclear structure is revealed especially clearly when luminescent and phase-contrast microscopy are combined.

Mesosomes (equivalent to mitochondria) have also been discovered in anthrax bacillus. These organoids are polyfunctional. The membrane-mesosome system in bacteria is not only responsible for oxidative phosphorylation, electron transport, and the di- and tricarboxylic acid cycles, but it is also involved in protein synthesis (G. I. Burd, 1967; Rose, 1968).

The mesosomes of vegetative anthrax bacillus cells are clearly outlined, bright yellow-green granules in direct contact with the cytoplasmic membrane.

Various inclusions are seen in the cytoplasm of vegetative anthrax bacillus cells. Volutin granules staining metachromatically may be found at the poles of the bacteria and sometimes at their center when they are stained by Loeffler's method, which involves holding the bacteria for some time in methylene blue solution. Staining with Sudan black reveals lipid granules, which are especially abundant during spore formation. They are encountered in spore-forming aerobic bacilli of all species, including among saprophytes.

Hochkiss' cytochemical reaction to polysaccharides reveals small glycogen granules. However, they are rarely encountered among both vegetative cells and bacteria initiating spore formation.

The Ultrastructure of Vegetative Microbial Cells

Roth and Williams (1963, 1964) were the first to report the submicroscopic structure of vegetative anthrax bacillus cells. Studying ultrathin spleen sections from mice infected by anthrax pathogen, they discovered elements of a discrete nucleoid in vegetative cells of this microbe.

The fine structure of virulent (No 66, No 2222) and vaccine (Tsenkovskiy's vaccine No 2, STI-1, Stern's) strains of anthrax bacilli had been studied in sufficient detail owing to the research of I. B. Pavlova and L. N. Kats (1966), A. A. Avakyan, I. B. Pavlova, L. N. Kats, and Ye. N. Levina (1967), I. I. Belokonov and G. V. Dunayev (1968), I. I. Belokonov (1970), T. A. Trzhetsetskaya and A. V. Kulikovskiy (1972), and Moberly, Shafa, and Gerhardt (1966).

The cell wall of anthrax bacillus seen in ultrathin sections is 360-400 Å, and it consists of three layers: two osmiophilic layers each 30-40 Å thick, and one osmiophobic layer 300-320 Å thick. However, this wall structure is not always noted. The wall consists more frequently of an internal osmiophilic layer of greater density and an outer, moderately dense layer. The outer layer of the wall often transforms into fibrillar structures that spread over the entire surface of the cell (Figure 2). It is hypothesized that these osmiophilic fibrillar formations are capsule remnants.

The cytoplasmic membrane is smooth or somewhat twisted. Its trilaminar structure can be revealed only in some portions of the cell. It is more noticeable in lysed cells. Usually its outer layer is in firm contact with the cell wall, giving the appearance of a single-layered formation (see Figure 2).

As a result of invaginational growth, the cytoplasmic membrane forms projections into the cytoplasm, differing in form, size, structure, and location, and described as intracytoplasmic membranous structures (mesosomes).

The thickness of the membranes in these formations is 80-100 Å. They are usually simple invaginations of the cytoplasmic membrane and appear as coils, ovals, and uneven lines. In many bacteria, the intracytoplasmic membranous structures penetrate into the zone of the nucleoid, and on occasion they are bound to it.

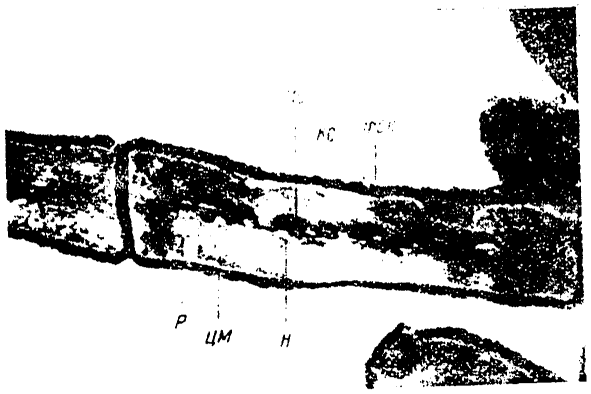

Figure 2. Ultrathin Anthrax Bacillus Section: КС--cell wall; ЦМ--cytoplasmic membrane; ФСК--fibrillar structures of the cell wall; МС--membranous structures; Н--nucleoid; Р--ribosomes. ×65,000.

The nucleoid appears as an extensive osmiophobic zone in the central part of the cell, and it is not sharply delimited from the cytoplasm. The bulk of the nucleoid consists of an irregular network of fibrils 25-50 Å thick distributed uniformly throughout its entire area.

The cytoplasm has a fine-grained structure, and it consists of 150-200 Å granules, similar in size to bacterial ribosomes. Ribosomes are often arranged in chains, forming polyribosomes. Clearly outlined vacuoles are encountered in the cytoplasm of many anthrax bacillus cells (Figure 3). They are often large, and enclosed by a membrane. Shape is imparted to the vacuoles by a membrane with polyribosomes on its outer side, which is especially noticeable in lysed cells. The vacuoles often concentrate near the nucleoid. In all probability the vacuoles form owing to dissolution of lipid inclusions, mainly poly-β-oxybutyric acid granules (Gerhardt, 1967), during fixation and dehydration.

Cell division involves formation of a transverse septum, as a result of which two individuals of equal size form. Creation of the transverse septum begins with invagination of the cytoplasmic membrane, with simultaneous inclusion of the cell wall in this process. As a result small symmetrically arranged identations oriented in the direction of the cytoplasm form.

The next act of division often begins before cells have separated following previous division. This leads to formation of streptobacilli. The length of individual bacteria in the chains often varies.

The ultrastructre of anthrax bacillus exhibits unique features during toxin biosynthesis and secretion when cultures are grown in special media (G. V. Dunayev, 1972).

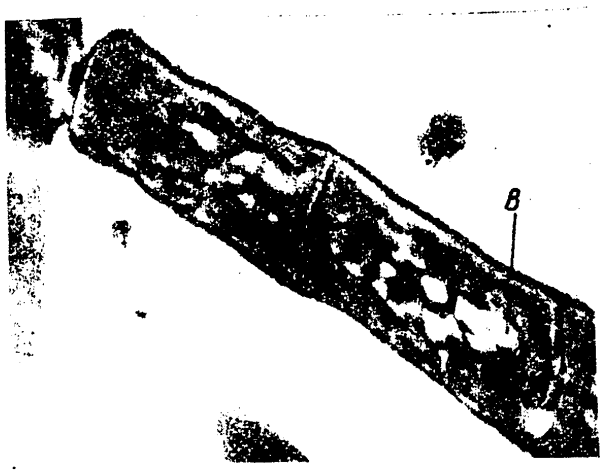

Figure 3. Ultrathin Anthrax Bacillus Section: B--vacuoles. ×55,000.

A culture in its exponential growth phase consists mainly of entire cells. The thickness of the cell wall of such bacteria is 330-350 Å, and the outer layer undergoes a transition into clearly pronounced fibrillar structures. The cytoplasmic membrane is a single-layered formation 80-110 Å thick with a convoluted outline. A high accumulation of osmiophilic masses can be noted within the zone of the nucleoid. Intracytoplasmic channels are revealed in some areas of the bacterial cells; they differ in their morphology from the usual type of membrane structures observed in microbes of this species: They are straight, short, and 110-120 Å wide, while membrane thickness is 75-80 Å. These channels pass through the cell wall and communicate with the outside environment.

Individual cells with a lysed protoplasm but with well preserved membrane structures are also encountered. Localized areas of lysed cell wall up to 375 Å in size are often revealed in such cells. Bacterial cells actively secreting toxin are observed in the exponential growth phase. (Figure 4). Inclusions of compact osmiophilic particles are noted in the cytoplasm of such cells.

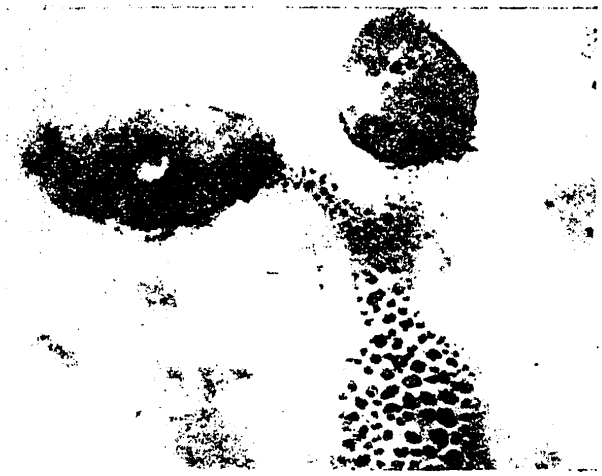

Figure 4. Ultrathin Sections of Anthrax Bacillus Cells Actively Secreting Toxin. ×39,000.

Outside the cells, the osmiophilic particles are contained in an optically less dense substance, also produced by secreting bacteria. As the particles get farther away from the microbial cells, they grow constantly larger, and the distance between them increases.

Intense development of the ribosome apparatus and the membrane-mesosome apparatus is noted among bacterial cells synthesizing toxin; a close relationship is simultaneously established between them, and intracytoplasmic membrane structures penetrate into the nucleoid zone.

When the microbial population is in its stable growth phase, cells are encountered with individual portions of the cell wall destroyed (local lysis), and it is through these breaks that a structure taking the form of fibrillar-granular osmiophilic material contained in the cytoplasm is secreted. There are no noticeable differences in the fine structure of vegetative cells of virulent and vaccine anthrax strains.

Microbe Staining Methods

Anthrax pathogen is stained intensively by alcohol-water solutions of the principal aniline dyes broadly employed in bacterial staining. Smears and impressions of the tissues of dead animals and blood smears stain very well with Loeffler's methylene blue.

Culture preparations may be stained in 1-2 minutes by a 1:10 dilution of fuchsin (Pfeiffer's alcohol-water solution) and Loeffler's methylene blue.

This bacillus (the vegetative form) reacts positively to Gram's stain. Negatively staining cells are encountered in very young and in very old cultures. This microbe does not have any specific tinctorial features inherent to it alone.

Capsule Formation

The Structure of the Capsule and Its Biological Significance

Anthrax bacillus forms capsules in the body of a sick animal or when it is grown in artificial nutrient media containing a large concentration of native protein. Intense capsule formation occurs during growth in both liquid and solid serum nutrient media.

In Gladstone-Fields medium, encapsulated rods appear after the third hour of incubation (A. V. Mashkov and V. P. Bodisko, 1958), and by the 14th-16th hours practically the entire culture consists of them. Then begins intensified diffusion of capsule substance from the surface of the cell into the surrounding medium.

GKI [State Control Institute of Biomedical Preparations] protein medium is an elective substrate offering the conditions for capsule biosynthesis by anthrax bacillus. The phenomenon of capsule formation is clearly noted in anthrax bacilli also in their growth in coagulated equine blood serum by Shaffer's method, as well as in serum agar, especially in a CO_2 atmosphere.

Capsule synthesis is rather intensively pronounced when capsule-forming strains are grown in protein media used to obtain protective antigen.

In this case capsule formation begins after 2.5 hours of growth, and it may be well pronounced in 6-hour cultures. Cells encased in a capsule are detected in 24-hour cultures as well.

However, in addition to encapsulated cells, bacteria devoid of capsules are also revealed in both the exponential and stable growth phases of virulent and capsule-forming vaccine strain cultures (Figure 5). This is evidence that mutants with different genetic characteristics, and mainly without the capsule glutamic acid polypeptide, are encountered in populations of different strains of anthrax bacillus.

In addition to native protein, an alkaline environment and presence of CO_2 promote capsule formation in culture (Sterne, 1937; Chu, 1952).

The influence of carbon dioxide upon the activity of some bacterial mitochondrial enzymes was established owing to research by Eastin and Thorne (1963). This research showed that the demand virulent and avirulent strains of anthrax bacillus exhibit for CO_2 during formation of capsule polypeptide differs: The former need it in higher concentrations, while the latter can also synthesize capsule polypeptide in an atmosphere devoid of CO_2.

Figure 5. Capsule-Forming Rods From a Protein Medium Culture: K--capsule. MBI-11. MNF-10. Objective 90 × 1.26; ocular photo 012.5x.

Encapsulated cells may be detected in the bodies of susceptible animals in just 2-3 hours after infection. However, at this time they can be found only in lymph nodes near the place of infection. When bacilli enter an immune organism, capsule formation apparently proceeds very slowly and extremely rarely.

A capsule synthesized by an anthrax microbe in an organism does not differ morphologically from a capsule produced by a bacillus grown in protein media, if we discount the fact that it is usually more massive in the latter case.

The capsule is more resistant to the action of putrefactive microflora than is the microbial cell. This is why only empty capsules can be found in material from decomposed carcasses of animals killed by anthrax. These empty capsules have come to be called microbial shadows.

A capsule is part of the cytoplasm synthesized by the microbial cell and excreted outside it. In this connection it is interpreted as a layer of ectoplasm surrounding the bacterium (Wildfuhr, 1959).

In ultrathin sections of capsule-forming anthrax bacilli taken from the organs of infected animals, the capsule is a thick, compact layer in close contact with the wall of the vegetative cell. The capsule of most microbes has a granular structure, and it contains clumps of osmiophilic material. Some bacteria retain only an inner fibrillar layer.

Encapsulated microbes in susceptible animals are surrounded by tissue detritus, and they are found in light zones formed as a result of the microbe's cytotoxic action (Figure 6).

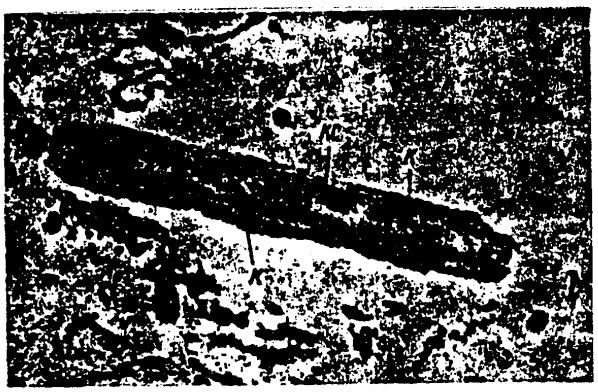

Figure 6. Ultrathin Section of an Encapsulated Rod From the Subcutaneous Tissue of a Dead Animal: K--capsule; KC--cell wall. ×48,000.

Three antigenic complexes were isolated in a study of capsule chemistry: capsule surface antigens (peptides apparently), antigens of the capsules themselves, represented by protein-polysaccharide substances, and cell membrane antigens, among which substances of polysaccaride and proteinaceous nature have been discovered (Ye. N. Levina and L. N. Kats, 1964; A. A. Avakyan, I. B. Pavlova, L. N. Kats, and Ye. N. Levina, 1967).

Studying the unique features of the glutamyl polypeptide antigen determinant of the membrane of anthrax bacilli, Scheiderka and Vacurik (1971) concluded that components other than peptides are not encountered in the capsule in this species of microbes. The component exhibiting the nature of a polysaccaride, discovered in serological reactions with anthrax-precipitating blood serum from a sick horse, is polysaccaride described by Ivanovich from the soma of *Bas. anthracis*. Thus it has not yet been established whether or not the anthrax bacillus capsule contains its own polysaccaride.

Tomcsik and Szongott (1933) were the first to establish that capsules produced by microbes of this species are devoid of reducing substances, that they do not contain native protein, and that up to 10 percent nitrogen is detected in them.

Somewhat later Ivanovics and Brucner (1937) found that a complex d-glutamic acid polypeptide is contained within the capsule substance of anthrax bacillus.

Capsule polypeptide produced by anthrax bacilli is interpreted as being one of the important factors of microbial aggression, since it is capable of suppressing the body's defensive phagocytic reaction. The capsule goes a long way in determining

the microbe's virulence (N. N. Ginsburg, 1964, 1966; Smith and Gallop, 1956), in which case the capsule polypeptide raises the activity of the lethal factor of extracellular anthrax toxin and simultaneously suppresses opsonization (Keppie, Harris-Smith and Smith, 1963).

Capsule Staining Methods

The anthrax microbe capsule stains best in preparations fixed with methanol (3-5 minutes), ethanol (10-15 minutes), and a mixture of equal volumes of ethanol and ether using Nikiforov's method (10-15 minutes). It also stains well in preparations fixed by the usual means with a burner flame. It should be remembered in this case that some of the vegetative cells do remain alive.

All negative bacterial capsule staining methods, including Burri's well known method, can be used to detect the capsule of anthrax bacillus. Nevertheless these methods are rarely employed: With all negative methods for studying capsules, the bacteria are not killed, and there is some danger in working with this microbe.

Anthrax bacillus capsules stain well in fixed preparations by all methods in use today: Mikhin's, Ol't's, Burtsev's, Romanvsky-Giemsa's, Johne's, Kaufmann's, Antoni's, Hiss's, Rebiger's, and so on.

Mikhin and Rebiger staining is especially effective. In Mikhin's method, a thick smear (predominantly from blood or from parenchymatous organs) is stained with Loeffler's methylene blue for 2-3 minutes during gentle heating (until vapor first appears). Then the dye is washed off quickly with a gentle stream of water, and the smears are dried on filter paper. Capsules are stained best with an old dye solution. Under the microscope, capsules appear light pink and the bacteria look dark blue.

Rebiger's staining method requires fixing the preparation with formalin simultaneously with staining. The following solution is used for staining: 50-20 gm gentian violet is dissolved in 100 ml 40 percent formalin; the solution is allowed to settle for several hours at room temperature, after which it is filtered. The unfixed smear is stained with this solution for 15-20 seconds, after which it is washed and dried. Capsules stain reddish violet, and bacteria stain dark violet.

Spore Formation

Spore Morphology and Ultrastructure

In conditions unfavorable to survival, anthrax bacillus is capable of forming typical spores.

Every vegetative cell, or sporangium as it is now called, forms only one spore, usually located centrally, and subterminally more rarely. Its diameter never exceeds the breadth of the bacillus. The spores very often lie separate from one another after the exosporium is destroyed. These spores are oval, sometimes rounded formations that refract light intensively. Mature spores are 1.2-1.5 μ long and 0.8-1 μ wide, and immature spores (prospores) are somewhat smaller (Figure 7).

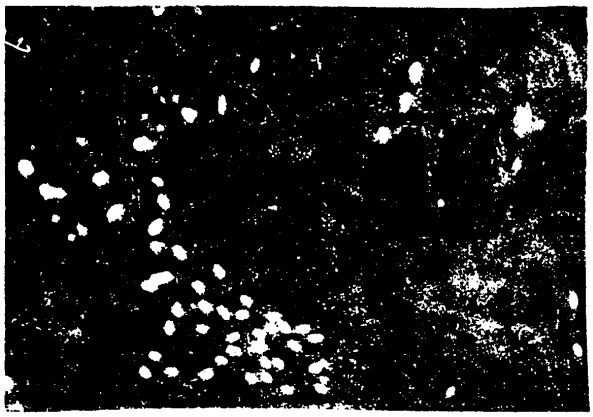

Figure 7. Anthrax Bacillus Spores: luminescent microscopy. ML-2. MNF-10. Objective 95 × 1.25; ocular photo 012.5x.

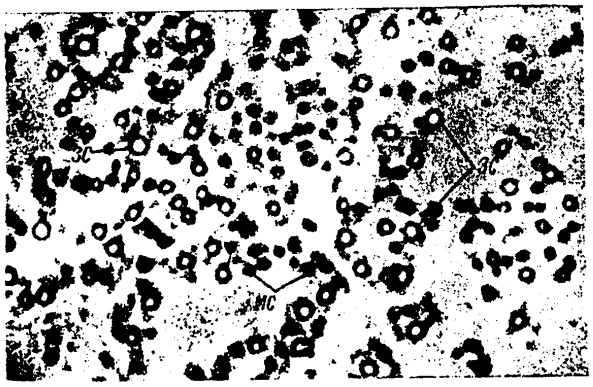

Figure 8. Spore Culture: 3C--mature spores; MC--young spores. Phase-contrast microscopy. ML-2. KF-4. MNF-10. Objective 90 ×1.25; ocular photo 012.5x.

Figure 9. Anthrax Spore Replicas: P--ribs. Electron microscopy. ×54,000.

The isolated internal part, which is in intimate contact with the outer dark layer, is readily evident in live mature spores (with phase contrast microscopy) (Figure 8). Younger spores and prospores are uniform and dark. When viewed with an electron microscope, mature spores stained with chromium or contrasted with phosphotungstic acid are optically impermeable formations with uneven outlines.

Ribs and, in some cases, fibers were discovered on the spore surface with the use of the hydrocarbon replica method suggested by Bradly and Williams (1957). The ribs orient themselves longitudinally or in honeycomb formation (Figure 9). The ribbed structure of the surface is more pronounced among spores from older cultures (15 days) than younger ones (I. I. Belokonov, 1970).

Presence of fibers on the surface of anthrax bacillus spores was first reported by Japanese and American authors studying the Akashi strain (Hachisuko, Koyima, and Sato, 1966) and the Stern strain (Moberly, Shafa and Gerhard, 1966).

I. I. Belokonov (1970) asserted the existence of fibers on spores of STI-1 and Tsenkovskiy vaccine No 2 vaccine strains.

G. V. Dunayev and I. I. Belokonov (1968) revealed parasporous corpuscles on spores of vaccine strain STI-1, which had been described earlier among some saprophytic spore-forming bacteria.

The paraporous corpuscles of anthrax bacillus have a regular spherical shape, and they are located on the surface of spores or apart from them (Figure 10). The diameter of the largest corpuscles attains 2,000 Å, that of moderate-sized corpuscles is 1,564 Å, while the diameter of small corpuscles is 1,200 Å. The biological significance of parasporous corpuscles on microbes of this species has not been determined.

I. I. Belokonov (1970) and T. A. Trzhetsetskaya and A. V. Kulikovskiy (1972) studied the ultrafine structure of the spores of virulent and vaccine anthrax bacillus strains.

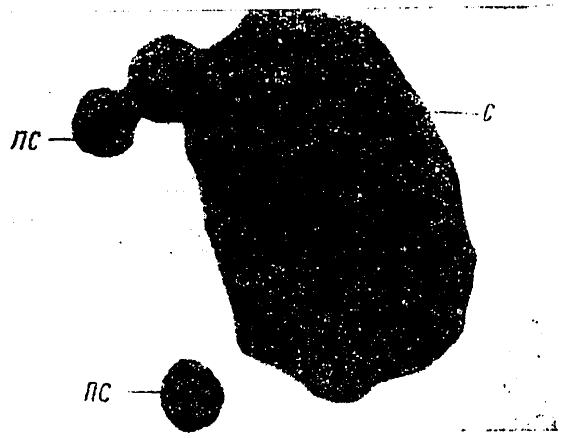

Figure 10. Spore and Parasporous Corpuscles: С--spore; ПС--parasporous corpuscle. Electron microscopy. ×64,000.

Formed spores have a multilayered sheath. In some areas up to six layers may be detected (Figure 11).

The cytoplasm of a spore (the sporoplasm) is surrounded by a membrane 150-200 Å thick (the cytoplasmic sheath).

The nucleoid is rarely detected, and it has the form of an indistinctly outlined zone of osmiophobic material. Intracytoplasmic membrane structures are detected even more rarely. The cortex is a massive, optically transparent layer 1,000-1,200 Å thick, in direct contact with the surface of the cytoplasmic membrane.

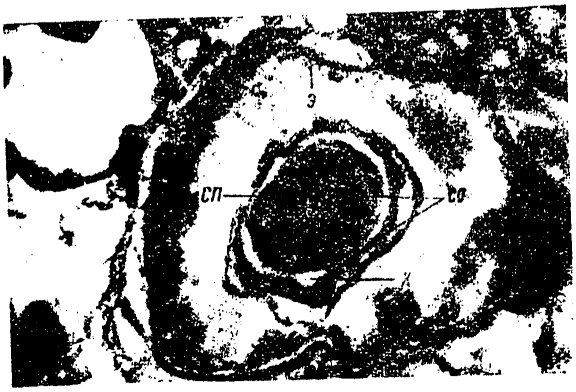

Figure 11. Ultrathin Section of a Spore of Virulent Strain No 2222: СП--sporoplasm; К--cortex; СО--spore sheaths; Э--exosporium. ×80,000.

On the outside, the cortex is covered by a two-layered spore sheath that attains a cross section of 300-350 Å. The outer layer of the spore sheath is surrounded by the exosporium, which is 150-200 Å thick; there are tiny fibers in some places on its surface.

The initial stage of spore formation in anthrax bacillus may be observed 18-20 hours after the medium is seeded and cultured at 37° (I. I. Belokonov, 1970; A. A. Avakyan, L. N. Kats, I. B. Pavlova, 1972).

The time of spore formation is governed by individual features of the strain, and it depends on temperature. There are strains exhibiting intensive spore formation in 12-14-hour cultures.

Spores form faster in solid than in liquid media, and as a rule the process is completed in 48-72 hours in readily sporulating strains grown in beef-extract agar. Spore formation does not occur below 15° and above 42°.

Bacillus anthrax spores seeded in beef-extract agar and maintained at 37° germinate after 1-2 hours: As a rule a 2-hour culture already consists of vegetative cells alone. Lyophilized spores germinate in about the same time as well; however, some spores require 7 hours to complete the process.

Vegetation time also depends on temperature; thus at 24° it lasts 16 hours, at 18° it lasts 70 hours, and at temperatures below 12° vegetation does not occur.

Spore germination requires amino acids as a source of nitrogen, carbohydrates (glucose is extremely necessary) for energy synthesis, and nucleic acid precursors. Germinating spores of anthrax bacillus require l-alanine and l-tyrosene (M. V. Zemskov, M. I. Sokolov, V. M. Zemskov, 1972).

Morphological changes in spores indicating the start of germination are recorded 5-10 minutes after the spores are seeded in nutrient medium (at 37°), and they are typified by swelling of the spores and appearance of small light areas along their periphery. Spores are released by rupture of part of the cell wall of the maternal cell. Cells do not germinate within the sporangium.

Spores contain alanine racemase, nucleoside ribosidase, and adenosine diaminase. In resting spores these enzymes maintain a low level of energy metabolism (respiration).

A shortage of proteinaceous substances in the nutrient substrate, insufficient moisture, a neutral or mildly alkaline pH, and absence of calcium promote faster spore formation. Sufficient aeration is mandatory.

It has been noted that growth and spore formation of anthrax microbes grown in an oxygen atmosphere proceeds more intensively than when the culture is aerated by atmospheric air alone, and that sporulation of vegetative cells is preceded by mineralization of the protein substrate. Spore formation begins the moment when the ratio of proteinaceous and mineral nitrogen in the medium shifts in favor of the latter (V. I. Yegorov and N. A. Spitsin, 1961).

This is why spore formation does not occur in a medium rich in proteinaceous substances, blood serum for example. The required ratio of the two forms of nitrogen is not achieved in these media.

Spores form in distilled water, in physiological solution, and even in an unfixed smear. Spores do not form in the living organism or in an undissected carcass, as well as in animal blood and blood serum.

Sporogenesis is suppressed by the addition of 1 percent calcium chloride solution to the medium; however, energetic spore formation is observed in the presence of neutral sodium oxalate (Bordet, Renaux, 1930).

When cultures are grown in nutrient media containing potassium bichromate and carbolic and hydrochloric acid, or when cultures are frequently reseeded in glycerin agar, asporogenic mutants of anthrax bacillus may be selected out. When the capability for spore formation recovers upon return of such artificially created asporogenic cultures into customary, normal conditions, formation of modifications of anthrax bacilli occurs in this case (phenotypic variability).

The biological role of spores can be described mainly as follows: They are a form of protection of spore-forming bacteria against the harmful influences of the environment, and thus they perform a survival function for the species. Spores are capable of preserving the material substrate of the initial cell's genetic information (the genome) for a long period of time, and of insuring transmission of the main characteristics to progeny in subsequent generations.

Spore Staining

Spores are not stained by the usual alcohol-water solutions of aniline dyes, since they enter into physicochemical reactions with various substances, including dyes, with difficulty.

When conventional staining methods are used, the spores lie colorless among stained vegetative bacterial cells. If phenol or heating is employed, staining does occur. Moreover in this case even acid is unable to remove the dye from resistant structures, though the vegetative part of spore-forming bacteria is rendered colorless quickly, and it may be restained any background color.

Anthrax bacillus spores may be stained by Aujeszky's, Zlatogorov's, Peshkov's, Moeller's, Trujillo's, Dorner's, Klein's and other methods. Aujeszky's and especially Trujillo's method is the most effective.

With Trujillo's method, smears fixed by a burner flame are dyed for 3 minutes with a saturated aqueous solution of malachite green while heating at the vapor point; then the dye is washed off with tap water, and the smear is restained for 1 minute with 0.25 percent fuchsin solution, after which it is washed and dried. The spores turn different shades of green depending on age, and vegetative cells turn red.

Anthrax pathogen spores may be revealed by luminescent microscopy.

Research by A. P. Kononenko and K. M. Ishchenko-Linnik (1960), V. M. Nikitina (1963), and Strugger (1946) showed that fluorochromed spores are seen much better with luminescent microscopy than spores stained by classical dyes.

G. V. Dunayev (1965) found the best conditions for separate fluorochroming of anthrax bacillus spores and vegetative cells, ones producing a clear microscopic pattern. In this method, smears fixed with a burner flame or with methanol for 5 minutes are processed according to the following procedure: *1*) 1 percent HCl solution is applied to the preparation for 1-2 minutes, and the glass is heated until vapor is given off; *2*) the preparation is washed with distilled water; *3*) carbolic auramine solution (a 1:1,000 solution of auramine in 5 percent chemically pure phenol) is applied for 10 minutes, during which time the glass is heated three or four times to the vapor point; *4*) the preparation is processed with 2 percent H_2SO_4 solution for 5 seconds; *5*) the preparation is washed with distilled water; *6*) the clarified preparation is additionally fluorochromed with rhodamine "6zh" (1:1,000) for 5 minutes; *7*) the preparation is washed with distilled water and air-dried.

When observed under a luminescent microscope, spores stained in this manner fluoresce a bright goldish-green color and they are distinctly outlined, while vegetative cells are bright red.

Culture Properties

Metabolic Features

One of the unique features of anthrax bacillus metabolism is its relationship to different forms of nitrogen. The lower the degree of hydrolysis of nutrient nitrogenous components, the more energetically this microbe assimilates nitrogen (V. M. Krasov, 1940). Anthrax bacillus also utilizes the products of radical protein hydrolysis.

A relatively small amount of amino acids is used in the vegetative reproduction stage, and bacteria develop predominantly due to destruction of more-complex protein complexes, which is in turn expressed outwardly as the capability for eliciting intensive proteolysis (V. I. Yegorov, N. A. Spitsyn, 1961).

Gladstone studied the amino acid demand of anthrax microbes back in 1939. He established that an anthrax bacillus culture grows quickly and luxuriantly in synthetic medium in the presence of leucine, valine, and isoleucine. Individually, however, each of these amino acids was toxic to anthrax bacillus and suppressed its growth. This property was expressed especially well with isoleucine. Gladstone concluded that not only are these amino acids capable of neutralizing the toxic action of one another, but they may also stimulate bacterial growth after the toxic effect is neutralized.

When leucine, valine, and isoleucine were absent, serine and threonine had toxic action when added to the medium separately. When added together, these amino acids neutralized the toxic action of one another. Three amino acids--leucine, valine, and isoleucine--exhibited different degrees of antagonism. In the presence of leucine and valine but in the absence of isoleucine, the bacterial culture did manage to develop in the end, despite an initial lag phase. Presence of valine and leucine in the medium was required so that the toxic action of isoleucine could be neutralized.

It was simultaneously demonstrated that leucine and valine are essential amino acids to anthrax bacillus, while isoleucine, glycine, and cystine may be synthesized independently by the bacterium following a certain initial lag phase.

These data on amino acid nutrition of anthrax bacillus agree with the results of more-recent studies by Smith and Tempest (1957).

Nutrient media containing protein complexes partially hydrolyzed down to peptones and polypeptides are the most favorable to growth of anthrax bacillus.

Nevertheless this bacterium may multiply intensively in synthetic nutrient media as well, for example in Wright's protein-free medium 528, which contains 17 amino acids. But the amino acids of such media must be selected with a consideration for excluding their mutual toxic action.

Anthrax microbe is classified in terms of its method of respiration as a facultative anaerobe. It develops well when exposed to heightened aeration, including an oxygen atmosphere (V. I. Yegorov and N. Ya. Spitsyn, 1961), and it exhibits growth in strictly anaerobic conditions.

M. Franke (1964) classifies anthrax bacillus as a strictly aerobic microorganism, and he believes that it may transform into a facultative anaerobe as a result of passages in nutrient media in which the oxygen concentration is gradually reduced. In these conditions it becomes asporogenic and Gram-negative.

Our numerous observations showed that during its first seedings in protein or protein-free media used to obtain protective antigen, anthrax bacillus grows unhindered in anaerobic conditions without losing its capability for positive Gram-staining (G. V. Dunayev).

As far as the demand for growth substances or vitamins is concerned, it has been established that only thiamine is necessary to normal growth and development of this microbe (Brewer, McCullough, et al., 1946).

Growth in Nutrient Media

The mineral composition of the medium has great significance to the vital activities of anthrax bacillus. As an example this microbe needs magnesium ions for normal development. Calcium and manganese do not have a noticeable influence upon its growth. The same can also be said for cobalt, zinc, copper, and lithium ions (Puziss and Wright, 1954).

Anthrax bacillus is not discriminatory in relation to nutrient media, and it grows well in universal media (beef-extract broth, beef-extract agar, beef-extract gelatin, potato, milk). It may be grown successfully on various substrates made from plant material: extracts of peas, soy, and vetch, slices of boiled potato, beets, carrots, and so on. The optimum temperature for growth in beef-extract agar and broth is 35-37°. At temperatures below 12° and above 45° anthrax bacillus does not grow. The optimum pH is within 7.2-7.6; however, culture growth is not inhibited in the pH range from 6.7 to 8.5, and it may even be observed at pH 6.0.

The first signs of growth may be noted as early as 3 hours after seeding on the surface of beef-extract agar or Hottinger's agar in aerobic conditions and at a temperature of 37°: 17-24 hour cultures consist of grayish-white fine-grained colonies with a silvery hue, resembling snowflakes. Colony diameter does not usually exceed 3-5 mm. Curls and braids consisting of long parallel strands diverge from the margins of the colony. The capability anthrax bacillus has for forming curls (ringlets) in solid nutrient media provided the grounds for comparing colonies of this microbe with the mythical head of Medusa or a lion's mane. Such colonies have a rough relief, they are characteristic of typical virulent strains, and they are referred to as the R-form.

When abundantly seeded, bacteria grow in the form of a continuous grayish-white incrustation, but the margins of this culture retain their curly nature. The spirals of the ringlets are directed toward the center of the colony.

On serum and blood agar as well as on Shaffer's coagulated equine blood serum, in an atmosphere containing 10-50 percent carbon dioxide, virulent strains grow in the form of smooth, semitransparent S-form colonies, and as mucoid M- or SM-forms (Stamatin, 1934; Sterne, 1937; Tacachachi, 1939; Chu, 1952). Such colonies consist mainly of encapsulated rods. Mutants unable to synthesize capsules produce rough R-form colonies in these media.

In beef-extract broth, Marten's broth, Hottinger's broth, vetch seed hydrolysate, and other liquid media, after 16-24 hours anthrax bacillus (the R-form) forms a loose white precipitate at the bottom of the test tube, while the supernatant remains transparent. When the test tubes are shaken, the broth does not become cloudy, and the precipitate breaks down into tiny flakes. A number of strains grow as separate delicate flakes suspended in the broth column; as a rule these clumps settle to the bottom in 24-48 hours.

Some weakly virulent strains and vaccine capsule-less strains, including Tsenkovskiy's No 2 vaccine strain, are capable of forming a loose ring around the test tube wall at the meniscus of the broth 2, 3, or 4 days after seeding; when the test tube is shaken gently, this ring breaks down into tiny flakes and settles. A film does not form on the surface of the medium.

Individual strains causing diffuse clouding of broth during growth are encountered in nature; when such cultures are shaken, they form delicate ripples.

Anthrax pathogen grows intensively in activated guinea pig, rabbit, equine, ram, and cattle serum, forming a luxurious flaky precipitate. Capsulogenic strains synthesize thick capsules in such media.

Anthrax bacillus grows highly characteristically when seeded by inoculation in a column of 10-12 percent gelatin. A yellowish-white rod forms on the 2d-5th days; delicate lateral outgrowths diverge from it at a right angle. These outgrowths are longer toward the surface of the medium (where aeration is better) and significantly shorter toward the bottom. Such growth recalls a fir tree turned upside-down. Gradually the upper gelatin layer begins to liquify, forming first into a crater and then a sack.

The microbe reproduces quickly in milk. It produces acid, and in 2-4 days it curdles the milk, after which the clot undergoes peptonization. On potato, it forms a luxuriant, dry, grayish-white coating which sometimes turns cream-colored.

Growth of anthrax bacillus in liquid synthetic media consisting of an assortment of amino acids, purine and pyrimidine bases, glucose, sodium bicarbonate, and inorganic salts, used to obtain protective antigen, hardly differs in any way from growth in beef-extract broth.

Anthrax bacillus also multiplies well in 8-12-day developing chick embryos. As they reproduce, the bacteria penetrate into the organs and tissues of the embryo, accumulating in significant quantities in the allantoic fluid. The embryos usually die 2-4 days after their infection. As the embryos increase in age, they become more resistant, even to infection by high culture doses.

Chemical Composition and Enzymatic Activity

Studying vaccine (Tsenkovskiy's No 1 and No 2 vaccines) and virulent anthrax pathogen strains, D. A. Tsuverkalov and V. M. Krasoz (1934) came to the conclusion that representatives of these strains hardly differ in any way from one another in relation to the quantitative composition of different forms of nitrogen. Nor were dramatic differences discovered in the concentration of amino acids, predominantly aromatic. As the virulence of anthrax pathogen increased, the concentration of lipoids in the bacteria increased while total phosphorus decreased. Cholesterol was not detected in the lipoids.

Dry residue from vegetative cells contains 6.8 percent nitrogen and 12-13.5 percent mineral ash; the corresponding figures for dry spore residue are 12.44 and 41.15 percent.

The quantity of DNA varies depending on the strain, and the highest level of RNA is recorded among bacteria in their exponential growth phase (Soru et al., 1952).

In biochemical respects anthrax bacillus is rather active; the following enzymes have been discovered in it: lipase, diastase, protease, gelatinase, dehydrase, cytochrome oxidase, peroxidase, catalase, arginase, etc. There is no urease,

phosphatase, or aspartase; nor is tryptophanase detected, as a consequence of which indole is not revealed in culture.

The bacillus ferments glucose, maltose, sucrose (slowly), trehalose, fructose (levulose), and dextrin, forming acid and no gas. Weakly pronounced acid formation, beginning at a later time and observed only among some strains, may be noted in media containing glycerin and salicin. Arabinose, rhamnose, galactose, lactose, mannose, raffinose, inulin, mannitol, dulcitol, sorbitol, and inositol are not fermented. Starch is hydrolysed. Citrates are utilized, and acetylmethylcarbinol is formed, owing to which a positive Voges-Proskauer reaction occurs. The bacillus synthesizes lecithinase (Bergey, 1957), and it slowly coagulates chicken egg yolk solution (M. V. Revo, 1958).

In contrast to anthrax pathogen, the spore-forming saprophytic aerobic bacterium *Bac. cereus* quickly coagulates chicken egg yolk solutions: It possesses extremely active lecithinase. It reduces methylene blue slowly, and it does not reduce nitrates and nitrites actively. It produces gelatinase as well as protease, and it hydrolyzes gelatin and coagulated serum rather quickly.

Proteolysis is frequently more active in virulent anthrax bacillus cultures than in weakly virulent ones; however, the saccharolytic properties of the latter are more pronounced (M. V. Revo, 1958).

Mutants are encountered in nature exhibiting insignificant residual virulence and high immunogenicity, ones that do not synthesize protease and do not cause liquifaction of protein substrates (nonproteolytic strains), for example the selected strains STI-1, Shuya-15, 1190-R, $34F_2$, Ikhtiman, etc.

D. A. Tsuverkalov (1955) studied the proteolytic activity of anthrax bacillus in relation to blood serum proteins (globulin, albumin) and caseine--milk protein. He established that both virulent and avirulent strains cause partial breakdown of protein, down to peptones are a rule. Decomposition of caseine and globulin proceeds in similar fashion, while transformation of serum albumin is unique, differing from breakdown of these other two proteins. It coagulates milk in 2-3 days, peptonizing it in the end.

Some strains split off hydrogen sulfide from peptones; this property is especially pronounced in media rich in peptones. This microbe forms ammonium.

Hemolysis proceeds extremely slowly in broth containing blood, and as a rule it does not occur in blood agar (N. A. Mikhin, 1942).

Anthrax bacilli do not usually hemolyse ram erythrocytes, or they lyse them very slowly; however, this characteristic is extremely variable among different strains (Ye. V. Gruz, 1964; N. N. Ginsburg, 1966; E. N. Shlyakhov, 1973; Seidel and Strassmann, 1956).

Irrespective of whether they are grown in the presence of carbon dioxide or in conventional conditions, virulent anthrax bacillus strains exhibit hemopeptic action, which is readily detected in Hottinger's agar containing 5 percent fresh ram blood, or in agar medium containing 15 percent defibrinated sheep blood.

Resistance

The resistance and survival time of vegetative cells and spores of anthrax bacillus vary. Cells are relatively labile, while spores are rather resistant.

Microbes in vegetative form in an undissected carcass undergo decomposition in response to proteolytic enzymes in 2-3 days; they may survive up to 4 days in buried carcasses; their lysis is completed even in bone marrow in 7 days (M. Franke, 1964). They die in the gastric juice of animals in 30 minutes at 38°, and they are able to survive 15 days in meat frozen at -15°, and up to 1.5 months in salted meat.

Anthrax bacillus spores are much more resistant and survive much longer in the environment than vegetative cells. Spore resistance depends in many ways upon how quickly the spores were formed. Thus spores formed at 18-20° are more resistant than spores formed at 35-38° (M. V. Revo, 1931). In certain cases spores may remain viable and virulent in the environment (in soil for example) for decades.

Resistance to Chemicals

Vegetative cells are not very resistant to various chemicals. Alcohol, ether, 2 percent formalin solution, 5 percent phenol solution, 1:1,000 mercuric chloride solution, 5-10 percent chloramine solution, fresh 5 percent calcium hypochlorite solution, and hydrogen peroxide lyse them in about 4-5 minutes.

Pathogen spores are more resistant. Ethyl alcohol at concentrations of 25 percent and higher kill spores in 50 days; spores are rendered harmless in a few days and even in the span of a few hours by 1:1,000 mercuric chloride, 5 percent phenol solution, and 5-10 percent chloramine solution, in 2 hours by 1 percent formalin solution, in 10-15 minutes by 2 percent formalin solution, in 15 minutes by 4 percent potassium permanganate solution, in 1 hour by 3 percent hydrogen peroxide solution, and in 2 hours by 10 percent caustic soda solution.

The effectiveness of decontamination depends on the temperature of the disinfectant, the spore concentration, and differences in the resistance of the strains. Moreover even microbes of the same strain vary in their resistance to disinfectants (A. A. Polyakov, 1964).

According to observations made by M. A. Sefershayev (1964) anthrax bacillus spores are resistant to resin phenols--waste products of shale industry. Three preparations in the interhalide compound groups--hydrochloric solution of iodine monochloride (preparations No 74 and No 74-B), piram, and niran--and one preparation in the chloro-active compound group--hypochlor--are active disinfectants possessing powerful bactericidal, sporicidal, and fungicidal action (G. M. Bosh'yan and T. A. Dmitriyeva, 1968).

Resistance to Physical Factors

Vegetative anthrax bacillus cells are not very temperature-resistant. When heated to 50-55°, they die in 1 hour; sometimes at 55° they undergo inactivation in as little as 40 minutes. They are inactivated in 15 minutes at 60°, in 1 minute at 75°, and instantaneously at the boiling point. Microbes are sensitive to dessication; however, spore formation occurs during slow drying, and the microbe does not perish.

On the other hand low temperatures preserve cells. Thus at -10° they survive 24 days, and at -24° they survive 12. Even at the temperature of liquid nitrogen (-196°), microbes do not die for 24 hours. Nevertheless at 2-4° they may die in as little as 2 weeks. Direct sunlight renders bacteria harmless in a few hours.

Spores are more resistant than vegetative cells. In general dessication does not have a lethal effect upon them. Spores in dried agar and gelatin cultures remain viable and virulent for 55 years (Hutyra, Marek, Manninger, Mocsy, 1959).

Dry heat (120-140°) kills spores in just 2-3 hours, at 150° they die in 1 hour, moving steam at 100° kills them in 12-15 minutes, and autoclaving at 110° kills them in 5-10 minutes. When boiled, spores die in 1 hour, while at a temperature of 400° they die in 20-30 seconds.

Direct sunlight destroys spores only after 4 days (M. Franke, 1964). Ultraviolet rays and X-rays have lethal action upon anthrax microbe spores: They cause their death in not less than 20 hours.

Resistance to Biological Factors

Anthrax pathogen is sensitive to lysozyme (L. N. Kats, 1964; A. A. Avakyan, I. B. Pavlova, et al., 1967; Gladstone and Johnston, 1955; Wright, 1961; Chatterjee and Williams, 1965). At pH 6.0-7.2 lysozyme breaks up anthrax bacillus chains, isolating individual bacteria and causing lysis of their cell walls, followed by formation of spheroplasts and protoplasts (Hordberg and Thorsell, 1955). As research by Perino (1960) showed, cellular respiration of anthrax bacillus changes in response to the action of lysozyme.

Freshly collected milk also has a bacteristatic effect. But its action is short-lived, only 24 hours, after which the bacilli begin to multiply and sporulate, retaining the pathogenicity inherent to them. The antimicrobial activity of milk is the consequence of lysozyme and lactins--enzymatic oxidation products (Kh. Kh. Abdullin and T. V. Kaparovich, 1971).

Growth of anthrax pathogen may also be retarded by fresh animal blood (V. V. Groman, 1884).

Somewhat later Buchner (1889) established that blood serum from animals of some species also has bactericidal properties. To a certain extent these properties are the consequence of the presence of special substances in serum--β-lysines (Peterson, 1936). Bloom, Watson, et al. (1947) isolated these substances from the tissues of various animals, giving them the same name--anthracocidins. According to observations of the authors, anthracocidins lyse entire bacterial cells, and they are not identical to lysozyme.

Anthrax bacillus is sensitive to the action of some antibiotics, mainly penicillin, streptomycin, oxytetracycline, tetracycline, and biomycin. The bacteristatic effect manifests itself both *in vitro* and *in vivo*. Minimum streptomycin concentrations retarding growth of anthrax bacillus vary within 1.15-2.34 μg/ml; the minimum oxytetracycline concentrations lie within 0.22-1.87 μg/ml.

The antimicrobial action of streptomycin and ocytetracycline differs depending on whether the antibiotics are used separately or in combination with one another: A mixture of streptomycin and oxytetracycline has more-pronounced action than do the antibiotics used separately; identical cumulative concentrations of these antibiotics, in micrograms per milliliter of medium, exceed the action of oxytetracycline by two times and the action of streptomycin by four times (V. M. Novikov, 1960). However, individual anthrax bacillus specimens resistant to antibiotics do exist in nature.

The Anthrax Phage

Back in 1898 N. F. Gamaleya established that anthrax bacilli undergo lysis *in vitro*. Gamaleya called the factor responsible for dissolution of the bacteria bacteriolysin; however, he did observe specific lysis of anthrax bacilli by an exogenous viral phage.

In 1922 Monterio published a communication stating that he had isolated a typical bacteriophage associated with anthrax pathogen. A specific phage was isolated from Tsenkovskiy's vaccine strain by Rozgon in 1929.

Active anthrax phages were subsequently isolated from effluent, animal feces, and old museum cultures by Cowles and Hale (1931), F. A. Terent'yev (1937), and A. P. Zotov (1940).

McCloy (1951) obtained a phage from an atypical *Bac. cereus* strain, which he named variant W and which was highly specific and was able to lyse each of 171 anthrax bacillus cultures he tested. Brown and Cherry (1955), Ivanovics and Lantos (1958), Stamatin (1959), A. Ya. Meshcheryakov (1961), Ya. Ye. Kolyakov and V. A. Bayrak (1962), Ye. V. Gruz (1964), V. S. Larina and L. S. Petrova (1964), and others isolated a number of phage strains from soil and lysogenic cultures. It was established that the phagolysis phenomenon may be utilized in practice for early differentiation of anthrax microbes from kindred aerobic spore-forming saprophytic bacilli. Clearly expressed phagolysis should be interpreted as an important orientational microbiological criterion of anthrax microbe identification.

Ye. N. Levina and V. R. Arkhipova (1967) studied anthrax phage from the comparative aspect in detail. They analyzed the morphology of negative colonies, heat resistance, adsorption rate in relation to different population sizes, lytic activity, and antigenic properties, and they analyzed the criteria of phage strain classification.

Six anthrax phages served as the object of study: VA-9 (Moldavian SSR Institute of Epidemiology and Hygiene), Saratov ("Mikrob" Institute), γ-Phage (Brown and Cherry), aC- and β-Phages (McCloy), and L_7-Phage (Stamatin).

There were four variants of the morphology of negative colonies, and they included all known types described for other bacterial phages. The authors subdivided the anthrax phages in their possession into two groups depending on antigenic structure: γ-phages and VA-9 phages (first group), and aC-, β-, and L_7 -phages (second group). This subdivision of the phages corresponded to their lytic properties. Phages in the first group possessed a broader lytic spectrum not only in relation to homologous microorganisms but also in relation to *Bac. cereus*.

As research by I. P. Pavlova showed (1973), phage "K" of the All-Union Institute of Experimental Veterinary Medicine possesses the broadest specific lytic spectrum.

The anthrax phage is a typical DNA-genome phage with a long noncontractile tail (A. S. Tikhonenko, 1968).

Anthrax Microbe Antagonists

That anthrax bacilli has antagonistic mutual relationships with other microorganisms was known in the past century.

Pasteur (1877) was one of the first to note that when anthrax microbes are introduced into the body simultaneously with other bacteria, development of the infe ious process does not occur.

Antagonism may be manifested both on the part of anthrax bacilli in relation to microorganisms of other species, and on the part of other microbes in relation to bacilli.

Anthrax bacillus suppresses the vital activities of *Bact. lactis*, *Staph. aureus*, *Bac. mycoides*, *Bac. subtilis*, *Mycobacterium luteum*, *Mycobacterium rubrum*, *Fusarium lini*, yeasts, and so on (Vaksman, 1947; N. A. Krasil'nikov, 1958; B. Ye. Ayzeman, 1973).

F. S. Shulyak (1974) established that anthrax microbes grown on solid nutrient medium produce a substance suppressing growth of *Pasteurella*.

However, the competitiveness of anthrax pathogen is not clearly pronounced, and in most cases it itself is subjected to inhibition owing to the antagonistic action of a number of microorganisms.

Clearly pronounced antagonistic influence upon anthrax bacillus is noted for *Escherichia coli*, *Salmonella gertneri*, *B. prodigiosum*, *Pseudomonas pyaceneum*, *B. ziguefasiens*, *Bac. mesentericus*, *B. aerogenes*, *B. proteus vulgaris*, *Staph. albus*, *Bac. cereus*, *Bac. mediosporus*, some species of streptococci, and others (Ye. N. Samodelkina, 1949; V. M. Vasil'yeva, 1959). *Bac. subtilis* may also play the role of anthrax bacillus antagonist, but as observations by V. M. Vasil'yeva showed, this property depends to a significant extent on the type of soil in which these two bacteria cohabitate.

Many putrefactive anaerobic bacilli and butyric bacteria of some species may also be interpreted to some extent as antagonist in relation to anthrax bacillus (K. A. Mirotvorskiy, 1940).

The antagonism of enterobacteria explains in part the rarity of intestinal forms of anthrax in animals (M. V. Revo, 1958).

According to observations by A. S. Korotich, A. D. Kalyuzhnaya, and G. Ya. Chuyskaya (1969) some species of actinomycetes, when introduced into the soil, have bactericidal action upon anthrax bacillus.

Pathogenicity

Mammals of all species are susceptible to anthrax pathogen. In natural conditions, sheep, goats, cattle, and horses are stricken most frequently, and asses and mules may be infected. Buffaloes, camels, and reindeer are susceptible. Pigs are less sensitive.

Cattle and whole-hoofed animals are infected most frequently by the pathogen when it enters the digestive tract than through the skin. On the other hand sheep are infected more readily by subcutaneous introduction of the pathogen. In order that such animals could be infected by the alimentary pathway, they must be given feed that would cause damage to the mucous membrane.

Dogs contract anthrax extremely rarely in natural conditions.

According to G. P. Sakharov (1908), dogs, especially puppies, may be infected by eating meat highly infested by pathogen spores. Cats are susceptible only when they are young (V. L. Yakimov, 1918).

Among wild animals, all herbivores (artiodactyls and perissodactyls) are susceptible. Reindeer are especially susceptible. Anthrax epizootics have often been recorded among moose. Anthrax outbreaks were noted among bison in 1962 in Canada's "Wood Buffalo" National Park (G. V. Kolonin, 1971).

There are reports of disease among roe deer, fallow deer, mouflon, wild boar, koodoo antelope, wart hog, zebra, giraffe (D. P. Pukhlyadev, 1943; E. N. Shlyakhov, 1960), and elephant (M. V. Revo, 1931). Sporadic cases of this disease are noted among elephants today in Burma, Thailand, and Liberia (M. G. Tarshis, 1971).

Anthrax is encountered significantly more rarely among predators, mainly among animals maintained in zoological gardens or circuses and eating infected meat. Sporadic cases of anthrax have been described among lions, tigers, leopards, panthers, pumas, cheetahs, wolves, polar foxes, sable, racoons, and bears, both in captivity and, in relation to some of the species listed above, in natural habitats (M. V. Revo, 1931; L. A. Rozen'yer, 1948; I. I. Kryuchkov, 1953; A. A. Sludskiy, 1954). Mink is extremely susceptible to anthrax.

Anthrax pathogen has been isolated many times by various researchers from rodents in the wild: the lesser, yellow, and sand suslik, the long-tailed marmot, the red-tailed and greater gerbil, and the wood mouse. The animals analyzed did not exhibit clinical disease; nevertheless cases of infection of some rodent species in natural conditions cannot be doubted (I. G. Galuzo, 1954; V. M. Tumanskiy, 1958; A. F. Nadzhafov, 1965; B. L. Cherkasskiy and L. M. Marchuk, 1971, etc.).

Anthrax was diagnosed among African ostriches, ducks, and eagles (P. M. Svintsov, 1951). The process is septicemic in ostriches and eagles.

Disease could not be evoked in birds by feeding them anthrax-infected material, though they did eliminate anthrax bacillus spores with feces, sometimes for a period of 30 days. Similar observations also exist in relation to mammals--foxes, coyotes, and jackals (P. N. Burgasov, B. L. Cherkasskiy, et al., 1970).

Chickens are rather resistant to anthrax, but they do not possess absolute immunity. Back in 1878 Pasteur and (Zhuber) managed to reproduce the disease in them after decreasing their body temperature by submerging their legs in cold water.

Reptiles, amphibians, fish, and invertebrates are unsusceptible. However, a frog may be infected if it is kept in water at 35°. Fish themselves do not fall ill, but they may be carriers of anthrax bacilli (V. L. Yakimov, 1918).

Cornelson, Toma, and Constantinescu (1950) infected turtles intracerebrally. They were able to isolate a culture of encapsulated bacilli from their brains.

The race and age of animals have a certain influence upon susceptibility. Thus the Algerian race of sheep and the East Indian ox are less sensitive to anthrax. Young animals are more susceptible than are old ones. Exhausted and starving animals are also more susceptible (Hutyra, Marek, Manninger, Mocsy, 1959).

While highly virulent strains may be isolated from soil, weakly virulent anthrax bacillus mutants may also be isolated from the carcasses of dead and sick animals. Their introduction into the animal body does not elicit typical disease.

Among laboratory animals white mice, guinea pigs, and rabbits are sensitive to anthrax. When these animals are infected with virulent strains, they develop acutely proceeding anthrax septicemia, terminating in death after 24-72 hours. Only white mice usually die after 24 hours. Guinea pigs and rabbits die on the 2d, 3d, and 4th days after infection. Animals die significantly more rarely on the 5th-7th days. Syrian golden hamsters, susliks, and polecats are sensitive to parenteral introduction of the pathogen.

White rats are practically unsusceptible; however, inbred rats of the Fisher-344 and Wistar lines are extremely sensitive to the toxin--the principal pathogenic factor of anthrax infection (Beal, Taylor, and Thorne, 1962; Bonvetre and Eckert, 1963).

Monkeys are sensitive to subcutaneous and inspirational infection. *Macaccus rhesus* is used for the most part for experimental purposes, and *Macaccus mulatta* is used more rarely (Darlow, Belton, Henderson, 1956).

White mice are infected by introducing the material subcutaneously in the rear part of the back at a 0.1-0.2 ml dose; guinea pigs and rabbits are also infected subcutaneously in the vicinity of the abdomen with a 0.3-0.5 ml dose of culture or of pathological material suspended in physiological solution.

After 15-22 hours rapidly increasing edema develops at the point of inoculation in guinea pigs and rabbits. Dissection reveals, at the place of edemic swelling, a gelatinous, colorless or slighly pinkish, readily movable moist mass, in which anthrax bacilli that are longer and less numerous than in blood, the spleen, the liver, and other organs are revealed.

Multiplying first at the point of introduction, the microbe surmounts the cellular and then the regional barriers (the lymph nodes), penetrates to the spleen and other organs, and finally it undergoes intensive reproduction in blood, attaining a density of $10^{8 \cdot 3}$ cells per mililiter of blood in guinea pigs (Klein, Mahlandt, Lincoln, et al., 1960). The infectious process develops similarly in other species of animals as well.

Variability

The moment anthrax bacilli came into being as an independent pathogenic species is not known. It is believed that during formation of normal microflora in the ungulate digestive tract, certain mutants of spore-forming bacilli, differing from the initial population in the spectrum of enzymes they contained, entered the tissues of the living organism through a damaged mucous membrane, survived, and reproduced. Viable progeny among them produced only those mutants that were capable of killing the hosts and entering the environment. In subsequent infections and exchanges of hosts, new properties (virulence first of all) necessary for further reproduction and survival of the microbe underwent consolidation in the genotype. Gaining a firm grasp on a new ecological niche with time, anthrax bacillus became an independent parasitic species which retained many traits in common with its saprophytic predecessors (G. V. Kolonin, 1970).

However, the question as to the origin of and the evolutionary ties of *Bac. anthracis* with other soil spore-forming bacilli, including *Bac. cereus*, remains debatable. It should be noted that no modifications of anthrax pathogen have ever been found in nature (N. N. Ginsburg, 1960).

Using the action of high temperature (42-43°) as a factor of selection in relation to virulent anthrax bacillus cultures, in the 1880's L. Pasteur and L. S. Tsenkovskiy obtained vaccine mutants from populations of this species of microbe, thus opening the first page of the teaching on the variability of anthrax pathogen.

The spectrums of the biological characteristics of different anthrax bacillus strains, both those isolated in natural conditions and those obtained in laboratory cultures, do not always coincide. Strains exhibiting diminished and heightened virulence, and asporogenic strains unable to synthesize a capsule or display hemolytic, strongly and weakly proteolytic, and saccharolytic properties in the organism and in protein-rich media are often isolated (M. V. Revo, 1958).

Dissociated forms have been determined sufficiently clearly for anthrax pathogen. It is well known that rough R-variants are virulent as a rule, that they possess pronounced residual virulence, and that they have typical morphological, cultural, and biological characteristics.

On the other hand the smooth, weakly virulent or avirulent S-variant is typified by cells existing in isolation. It causes uniform clouding and produces an amorphous precipitate in broth, and smooth colonies with even margins in agar.

Numerous reports have been published on the variability of anthrax microbe in natural and laboratory conditions. Changes have been described in morphology, in tinctorial and cultural properties, and in virulence, and nonencapsulated and asporogenic strains have been isolated. Without a doubt, these were selected mutants or variants of phenotypic variability (modification).

Penicillin was used as the factor inducing phenotypic variability in STI-1 vaccine strain in experiments conducted by A. N. Smirnov and S. N. Preobrazhenskiy (1965). This antibiotic disturbed cell wall formation and caused the appearance of the L-form.

We observed formation of unstable L-forms of anthrax bacilli in response to lysozyme contained in guinea pig blood serum and in response to a temperature of 45°; when these two factors were combined, cell wall synthesis was inhibited. Elimination of the inducing factor was accompanied by total reversion to initial forms.

Investigation of the variability of anthrax bacilli led to creation of highly effective capsule-less vaccines used for immunization of animals. These include strains 1190-R (Stamatin, 1934), $34F_2$ (Sterne, 1937), STI-1 (N. N. Ginsburg, 1942), and Shuya-15 (S. G. Kolesov et al., 1946-1949, 1955).

BIBLIOGRAPHY

1. Abdullin, Kh. Kh., and Kaparovich, T. V., "Aktual'nyye voprosy profilaktiki sibirskoy yazvy v SSSR" [Pressing Problems of Anthrax Prevention in the USSR], Moscow, 1971, p 145.

2. Avakyan, A. A., Pavlova, I. B., Kats, L. N., and Levina, Ye. N., ZHMEI, Vol 11, No 2, 1967, p 140.

3. Avakyan, A. A., Kats, L. N., and Pavlova, I. B., "Atlas anatomii bakteriy patogennykh dlya cheloveka i zhivotnykh" [Atlas of the Anatomy of Bacteria Pathogenic to Man and Animals], Moscow, 1972.

4. Belokonov, I. I., and Dunayev, G. V., "Sibirskaya yazva v SSSR i perspektivy yeye likvidatsii" [Anthrax in the USSR and the Prospects of Its Eradication], Moscow, 1968, p 57.

5. Burgasov, P. N., Cherkasskiy, B. L., Marchuk, L. M., and Shcherbak, Yu. F., "Sibirskaya yazva" [Anthrax], Moscow, 1970.

6. Ginsburg, N. N., "Antraks" [Anthrax], Kishinev, 1964, p 4.

7. Dunayev, G. V., SB. VETERINARIYA, Kiev, Vol 7, 1965, p 143.

8. Dunayev, G. V., SB. VETERINARIYA, Kiev, Vol 12, 1967, p 88.

9. Yegorov, V. I., and Spitsyn, N. A., VETERINARIYA, Vol 2, 1961, p 45.

10. Kats, L. N., MIKROBIOLOGIYA, Vol 33, No 5, 1964, p 836.

11. Kolesov, S. G., TRUDY GNKI, Moscow, Vol 5, 1955, p 12.

12. Kolonin, G. V., ZHMEI, No 1, 1971, p 118.

13. Krasov, V. M., VETERINARIYA, No 4, 1940, p 3.

14. Levina, Ye. N., and Kats, L. N., ZHMEI, No 10, 1964, p 85.

15. Mikhin, N. A., "Sibirskaya yazva u cheloveka i sel'skokhozyaystvennykh zhivotnykh" [Anthrax in Man and Agricultural Animals], Moscow, 1942, p 20.

16. Pavlova, I. B., and Kats, L. N., ZHMEI, No 2, 1966, p 90.

17. Polyakov, A. A., "Veterinarnaya dezinfektsiya" [Veterinary Disinfection], Moscow, 1964.

18. Revo, M. V., "Kurs spetsial'noi mikrobiologii" [A Course in Special Microbiology], Khar'kov-Kiev, 1931.

19. Revo, M. V., and Zhukova, M. A., "Veterinarnaya mikrobiologiya" [Veterinary Microbiology], Moscow, 1958.

20. Tarshis, M. G., "Geografiya bolezney zhivotnykh zarubezhnykh stran" [Geography of Animal Diseases of Foreign Countries], Moscow, Izd-vo "Kolos", 1971, p 64.

21. Terent'yev, F. A., "Sibirskaya yazva" [Anthrax], Moscow, 1940, p 53.

22. Trzhetsetskaya, T. A., and Kulikovskiy, A. V., "Vsesoyuznyy NII vet. sanitarii. Problemy vet. sanitarii" [All-Union Scientific Research Institute of Veterinary Medicine. Problems in Veterinary Medicine], Moscow, 1972, p 5.

23. Franke, M., "Infektsionnyye bolezni" [Infectious Diseases], Bucharest, Vol 2, 1964, p 693.

24. Shlyakhov, E. N., "Epidemiologiya, diagnostika i profilaktika sibirskoy yazvy" [Epidemiology, Diagnosis, and Prevention of Anthrax], Kishinev, 1960.

25. Bloom, W. L., Watson, D. W., Cromartie, W. J., and Freed, M., J. INFECT. DIS., Vol 80, No 1, 1947, p 41.

26. Brown, E. R., and Cherry, W. B., J. INFECT. DIS., Vol 96, 1955, p 34.

27. Chatterjee, B. R., and Williams, R. P., J. BACTERIOL., Vol 89, No 4, 1965, p 1128.

28. Chu, H. P., J. HYG., Vol 50, 1952, p 433.

29. Gladstone, G. P., and Johnston, H. H., BRIT. J. EXPTL. PATHOL., Vol 36, No 4, 1955, p 336.

30. Hutyra, Marek, Manninger, R., and Mocsy, J., "Spezielle Pathologie und Terapie der Haustiere", Jena, Vol 1, 1959.

31. Ivanovics, G., and Lantos, J., ACTA MICROBIOL. HUNG., Vol 5, 1958, p 405.

32. Keppie, J., Harris-Smith, R. W., and Smith, H., BRIT. J. EXPTL. PATHOL., Vol 44, No 4, 1963, p 446.

33. Klein, F., Mahlandt, B. G., Lincoln, R. E., DeArmon, I. A., and Fernelius, A. L., SCIENCE, Vol 133, No 3457, 1960, p 1021.

34. McCloy, E., J. GEN. MICROBIOL., Vol 5, 1951, p 16.

35. Puziss, M., and Wright, G. G., J. BACTERIOL., Vol 68, No 4, 1954, p 474.

36. Smith, H., and Gallop, R. C., BRIT. J. EXPTL. PATHOL., Vol 37, No 2, 1956, p 144.

37. Smith, H., and Tempest, D. W., J. GEN. MICROBIOL., Vol 17, No 3, 1957, p 731.

38. Sterne, M. Onderstepoort, J. VET. SCI. ANIMAL INDUST., Vol 8, No 2, 1937, p 271.

39. Tomcsik, J., and Szongott, H., Z. IMMUN.-FORSCH., Vol 77, No 1/2, 1933, p 86.

40. Wildfuhr, G., "Medizinische Mikrobiologie, Immunologie und Epidemiologie," Vol 1, Leipzig, 1959.

41. Wright, G. G., PROC. SOC. EXPTL. BIOL. MED., Vol 108, No 3, 1961, p 740.

EPIZOOTIOLOGY

Historical Survey

Anthrax has been known since ancient times. It was studied by Arabian, Greek, and Roman physicians.

Anthrax was especially widespread among animals and people in the Middle Ages. During this time various speculative theories on the cause of such epidemic diseases appeared. Included among them are the miasmatic and contagious theories.

According to the first theory epizootics and epidemics were elicited by an infectious agent, miasma, arising in air, in soil, and in water. Penetrating into the bodies of people and animals, miasma elicits infectious disease, including anthrax, which was what that disease was called by that time.

According to the contagious theory infectious diseases were elicited by a contagium arising in the animal body. This infectious agent could be transmitted from one person or animal to another. The Finnish scholar Solander asserted that anthrax develops in horses after they are bitten by a "particular" animal carried aloft by the air. Its description was so "persuasive" that even such a prominent taxonomist such as Carolus Linnaeus introduced this "animal" into his system as "*Furia infernalis*"--the fury of Hell.

The first to approach clarification of the causes of anthrax infection from a scientific point of view was the Russian physician S. S. Andreyevskiy, who demonstrated in 1788, through a heroic self-infection experiment, that the disease affecting man was identical to that affecting animals, and thus he resolved the issue of the disease's nosological independence. Introducing blood from sick animals into healthy sheep, in 1836 the German researcher (Eylert) induced a disease similar to anthrax in them.

Discovery of this infection's pathogen was a major step forward in the study of anthrax. Tiny nonmotile strand-like bodies were discovered in the blood of animals killed by anthrax by Pollender in Germany in 1849 and by Davaine and Rayer in France in 1850. They did not attach etiological significance to the bacilli, believing them to be an incidental discovery. In 1857 F. Brauel' found, in the blood of animals and a person that had died from anthrax, the rods noticed by Pollender, Davaine, and Rayer. The investigator infected experimental animals with blood from fallen and sick animals. He turned his attention to the fact that the rods were not present in the blood of fetuses in pregnant animals suffering anthrax, but that they were present in the blood of the mothers.

Brauel' studied the infectiousness of the blood of animals suffering from and killed by anthrax on animals of several species. He determined the incubation period, which according to his data varied from 44 to 78 hours, he established that animals of different species vary in their susceptibility to this infection, and he noted that the rods discovered in the blood have diagnostic significance (E. Pebsen, 1958).

A great contribution was made to the study of anthrax epizootiology by V. F. Nagorskiy, S. N. Vyshelesskiy, N. A. Mikhin, A. A. Vladimirov, M. S. Gannushkin, R. A. Tsion, F. A. Terent'yev, and many other researchers.

Origin, Evolution, and Spread of Anthrax Infection

V. A. Kraminskiy and Yu. I. Sorkin (1970) classify anthrax as one of the diseases for which the pathogen had formerly inhabited the outside environment and later adapted itself to obligate parasitism in animals. The term "obligate" is not entirely suited to the anthrax pathogen. This microorganism also develops well on various nonliving organic substrates, and soil and small water basins serve as its habitat. Aerobic bacilli are also active components of the soil ecosystem. Among them, there are many with morphophysiological and antigenic properties highly similar to those of *Bac. anthracis*. For example, *Bac. cereus* possesses some pathogenic and toxigenic properties (Nordberg, 1953; K. A. Akhundova, 1967). On this basis some authors (V. M. Zhdanov, 1964; V. A. Kraminskiy, Yu. I., Sorokin, Brawn, 1955) believe it to be the ancestor of *Bac. anthracis*, while others (Smith, Gordon, Clark, 1952), assert on the contrary that *Bac. cereus* transformed out of *Bac. anthracis* owing to the latter's loss of its pathogenic properties.

It has been established by experimental research that *Bac. anthracis* acquired the characteristics inherent to *Bac. cereus*. We established that in response to the action of essential oils from various plants, *Bac. anthracis* loses its pathogenecity and acquires all properties inherent to *Bac. cereus*, except for formation of cilia.

The following ideas could be suggested concerning anthrax pathogen's acquisition of parasitic and pathogenetic traits. Eating plants, herbivorous animals (especially ruminants) could have injured the mucous membrane of the oral cavity and the esophagus. The ancestors of anthrax bacillus--soil microbes--could have penetrated into the host's body through these openings. As a result of many such contacts a mutant form of the microbe, capable of capsule formation in the body, might have arisen.

Subsequent selection leading to acquisition of pathogenicity by the capsule-forming microbe while in the body could have proceeded in the direction of production of toxic metabolites. It has now been established that the pathogenicity of anthrax bacilli is the product of their capability for forming capsules and producing complex three-component toxins (Smith, Keppie, 1955, G. V. Dunayev, 1972; Yu. V. Yezepchuk, 1968).

Several hypotheses have been suggested concerning the evolution of the epizootic process of anthrax. G. V. Kolonin (1970) presumes that anthrax arose as an infection prior to the advent of man, and that it was a disease of wild ungulates--that is, it was a natural focal infection, the pathogen of which was a member of the natural ecosystem. In the author's opinion the origin and arisal of anthrax

as an infection could be attributed to the beginning of the Quaternary period. This period of the organic world's evolution offered all of the conditions for manifestation of the epizootic process of anthrax. Enormous herds of cloven- and whole-hoofed animals susceptible to this infection grazed the boundless open plains. In these conditions, it is assumed, more-virulent individuals underwent further selection within the pathogenic population. Domestication of wild ungulates by man, which began in the neolithic era, resulted in the spread of the range of anthrax, and intensification of the epizootic process. Its range was extended to countries of the Mediterranean coast prior to our era (O. V. Baroyan, 1968).

Importation of the pathogen into new territories occurred in connection with progress in human society's acitivity and greater intensity of communication among nations. According to Sh. Nikol' anthrax was carried with domestic animals into Central and South America in the 16th-17th centuries, and into North America in the 17th century. The disease was carried into the tundra from the taiga of the former Arkhangel'sk Province in the 17th century at the earliest (G. V. Kolonin). In the 19th century it was imported into Australia, Cumberland County, with bone meal (Nokar and Leklansh, 1897).

Anthrax is now encountered on all continents of the globe. Anthrax has not recently been noted in the tundra of North America, Scandinavia, and the northeast USSR, as well as on a small number of island territories. It is significantly widespread in Spain, Italy, Albania, Greece, Turkey, Iran, Syria, India, Burma, Indonesia, Kenya, Uganda, Tanzania, Rwanda, the UAR, Mexico, El Salvador, Guatemala, Costa Rica, Argentina, Chile, Paraguay, and Brazil. It is observed as sporadic cases in Norway, Sweden, Denmark, Finland, Ireland, Canada, and Australia.

Anthrax was extremely widespread in prerevolutionary Russia, especially before the vaccine was discovered (Table 1).

Table 1. Incidence of Anthrax Among Animals From 1885 to 1912 in Russia and From 1920 to 1923 in the RFSFR (Thousands) (From N. A. Mikhin, 1942)

Year	Incidence	Year	Incidence	Year	Incidence	Year	Incidence
1885	33.5	1893	27.7	1901	67.7	1909	44.2
1886	16.6	1894	16.4	1902	53.3	1910	45.2
1887	21.2	1895	27.7	1903	58.6	1911	44.0
1888	29.9	1896	59.1	1904	36.0	1912	43.2
1889	39.7	1897	54.0	1905	32.7	1920	30.0
1890	100	1898	146.5	1906	60.4	1921	86.0
1891	47.5	1899	46.5	1907	49.7	1922	26.8
1892	37.3	1900	40.6	1908	42.4	1923	

The years 1890, 1898, and 1901 were especially unfavorable. Droughts and associated crop failures and famines were typical of these years.

A tendency toward lower incidence of anthrax among farm animals was observed later on. Making a comparative analysis of morbidity statistics for 1890-1899 in different geographic zones of European Russia, and for 1961-1970 in the same zones in the USSR, S. I. Dzhupina (1973) established a decline in the incidence of anthrax among animals by a factor of 278.

Susceptibility of Animals

Deer are the most susceptible to anthrax. Then follow, in order of decreasing susceptibility, sheep, goats, cattle, horses, bison, antelope, buffalo, pigs, and camels. Sable, mink, American raccoon and marten are especially sensitive, while cats, dogs, wolves, foxes, Ussuriysk raccoons, and polar foxes are weakly susceptible. Deaths among bears, elephants, lions, tigers, and jaguars have been described.

A difference has been noted in the anthrax susceptibility of animals of different races in the same species. As an example Algerian sheep are more resistant than sheep of European races. According to N. M. Noskov (1958) cows of domesticated races (Tagil', Istobensk, Yaroslavl', Schwyz, Simmental, and others) were the principal ones to be infected by anthrax in the Omsk and Barbinsk districts during the epizootic of 1929, while aborigine and Red Steppe breeds were infected extremely rarely.

The literature contains very little data on the susceptibility of different ages of agricultural animals. Nokar and Leklansh (1897) note that young animals generally get anthrax more frequently. The young even of animals having natural specific immunity when mature are susceptible. It is possible that adult animals possess special protective factors absent from newborn animals.

The reverse pattern is observed with naturally susceptible animals: The young are more resistant to anthrax infections than adult animals. N. M. Noskov (1958) established on the basis of anthrax epizootic statistics and experimental research data that calves up to 1 year old do not get anthrax in natural conditions. When immunized with Tsenkovskiy's anthrax vaccines, they do not exhibit a visible general or local reaction prior to 6 months of age, and it is only among some that are 6.5-7 months old that limited, quickly-passing swellings appear. The pronounced bactericidal nature of the blood serum of calves in the first months of postnatal development and the high phagocytic activity of leukocytes in relation to Tsenkovskiy vaccine No 1 microbes attest to the great biological significance of nonspecific immunity factors against anthrax in calves.

Of certain interest in this aspect are observations by reindeer herdsmen and veterinarians noting that while almost 100 percent of adult animals die of anthrax, young reindeer are immune. S. N. Vyshelesskiy and S. I. Rasputin (1916) were among the first to report this. A similar pattern was observed by L. D. Nikolayevskiy (1936). Ye. M. Borisov, A. V. Orlov, and M. A. Tolokonskiy (1933) noted that the calves that do die are sometimes not even ones which had nursed from mothers that had died of anthrax.

V. P. Slentsov, a veterinarian of the Yakut ASSR (Republic Veterinary Bacteriological Laboratory), reports that during the 1969-1970 anthrax outbreak in Olenenskiy Rayon of the northwestern Yakut ASSR, 15 percent of the adult reindeer in one herd of Olenensk Sovkhoz died in 3-4 days. Seventy 2-month fawns grazed in the infected areas, and from time to time they attempted to nurse from mothers that were in an

agonal condition or had already died. These fawns were kept under observation for 17 days, after which they were herded to other pastures with the rest of the herd. Cases of disease among the calves began to be recorded from 10 June to 12 July. Judging from the time the animals fell ill, the possibility that the fawns were inoculated with anthrax agent by horseflies is not excluded.

Different opinions have existed until recently on the resistance of young sheep to anthrax. The first to turn his attention to the resistance of lambs to anthrax was Chauveau (1880). He infected Algerian lambs following their birth and, simultaneously, their four mothers. Of the ewes, one died, while all lambs remained alive. Toussaint (1883) infected lambs born of ewes vaccinated prior to pregnancy. These lambs did not fall ill in their first month of life. It follows from a report by N. I. Ekkert (1891) that out of 10 unvaccinated lambs experimentally infected (the age is not indicated), 7 died.

Other authors have concluded, from experiments conducted on small numbers of animals or from observations of the course of disease, that lambs are more susceptible to anthrax than adult sheep, or that they are identically resistant to this infection (A. P. Shalashnikov, 1888).

At the same time practicing veterinarians noted that during epizootics, the young were less susceptible to anthrax than were the adult animals. The resistance of the young did not always depend on whether or not the ewes had been vaccinated.

Lambs 2-3 months old in experiments performed by G. I. Romanov (1969, 1971) were more resistant to artificial infection than were adult sheep. Thus following infection of animals at a dose of 25,000 spores, 30 percent of 10 lambs 2.5 months old and 20 percent of 10 lambs 3.5 months old survived, while out of 5 lambs 4.5 months old, all 5 died. Fourteen out of 15, or 93.3 percent, of sheep infected by the same dose died. The experiments showed that there is a significant difference between survival of lambs 2-3.5 months old and adult sheep infected by identical doses of a virulent culture of anthrax pathogen.

The following data are available concerning the susceptibility of the young of other animal species to anthrax. As an example G. P. Sakharov, who performed experiments on guinea pigs, white rats, rabbits, and dogs of different ages, concluded that young animals are more susceptible to anthrax than are adults. However, the author's experimental data do not permit this conclusion relative to animals of the first three species, inasmuch as both the young and the sexually mature individuals died after being infected with virulent anthrax culture. The only difference was that the older animals died a few hours later. As far as dogs are concerned, the author's experiments confirm that puppies are more susceptible than their mothers.

However, performing experiments on adult dogs and puppies 27-30 days old, N. N. Bogdanov (1948) established that when maintained under ordinary conditions, adult dogs and nursing puppies do not fall ill following infection by bacillar and spore forms of anthrax pathogen culture introduced both perorally and subcutaneously. Piglets, as well as newly-hatched chicks, are infected by anthrax relatively easily (Nocard, Leclainche, 1906).

Kh. Kh. Abdullin, Sh. Kh. Kharisov, D. Sh. Akhmerov, and M. S. Yezhkova (1973) fed 3-4 month old kids the milk of goats that had fallen ill and recovered following artificial infection by a spore suspension of anthrax microbes.

The initial pathogen culture was isolated from the milk. The milk was fed to the kids for 2-16 days. During this period different animals received from 320 gm to 6.5 liters of milk. Of 20 kids, not a single animal contracted a clinically pronounced form of anthrax. During the experiment the animals were killed and analyzed. Capsule forms of anthrax bacillus were discovered in mesenteric lymph nodes and in parenchymatous organs of 13 out of the 20 kids. We explained the negative infection results by the method used to introduce the microbe.

Thus the impression is created that age-related differences in sensitivity to infection exist among mammals susceptible to anthrax. A certain degree of resistance is noted among newborn animals and during the nursing period; resistance subsequently declines to a constant level which is maintained until the animal attains maturity. From this time on, resistance declines further.

Individual differences also exist in susceptibility to anthrax infection exhibited by a population of animals of a particular species.

As an example V. F. Nagorskiy (1902) notes that the reports of commissions of the Veterinary Committee testing the action of anthrax vaccination in Russia in 1887-1889 contain descriptions of cases in which unvaccinated animals used as controls--sheep, cattle, and horses--did not fall ill after being given a highly virulent culture of anthrax pathogen not only through the mouth (this resulted in disease only rarely), but also subcutaneously at doses up to 0.5 ml.

Statistics cited by L. P. Levitskiy and V. F. Nagorskiy on anthrax epizootics in the era prior to vaccination show that the largest number of animals in an enzootic focus fall ill in the first 2 weeks of infection. The decrease in morbidity cannot be explained by just arisal of immune head as a result of accumulation of reconvalescents, or animals that had suffered a latent form of infection--immunizing subinfection using R. F. Sosov's term (1973). Significant accumulation of immune head within a population of animals occurs in later periods of an epizootic. This phenomenon should also be interpreted as inclusion of more-susceptible individuals at the beginning of the epizootic process.

Kh. Kh. Abdullin, Sh. Kh. Kharisov, and D. Sh. Akhmerov observed individual differences in the resistance to anthrax pathogen exhibited by artificially infected goats and cows. The animals were infected by a spore suspension of anthrax microbes prepared by the VGNKI [not further identified]. A goat infected by a dose of 1,000 spores died 96 hours after infection, while goats administered 50,000 spores survived. The difference was rather significant.

The result of infecting the cows is more indicative in this regard. Of two cows infected by a dose of 25 billion spores, one died 63 hours after infection, while the other remained alive. A cow receiving 50 billion spores also survived. Two cows infected with a dose of 1 billion spores suffered the acute form; two cows--one infected by 8 billion spores and the other by 2.3 billion--suffered the mild form (abortive form). In view of the small number of animals in the experiment, these data cannot be treated as exhaustive.

Arisal and Course of Epizootics

The arisal of any infectious epizootic depends on a number of factors and conditions. These factors are extremely multifaceted in relation to anthrax infection, but the following are the basic determinants: the source of the infectious pathogen, the pathogen transmission pathways, and presence of a sufficient number of susceptible animals. These factors make up the epizootic chain.

Development of an epizootic requires this chain to be continuous.

As with all other infections, the source of anthrax infection is sick animals exhibiting different courses of infection. When the course of disease is clearly apparent, the pathogen is liberated into the environment from the body of the sick animal with urine, feces, saliva, and milk.

Until recently, little has been said in the special literature about the significance of sick animals with abortive and latent forms of disease and of reconvalescents as the source of anthrax infection. The atypical abortive form of anthrax infection is not a rare phenomenon among agricultural animals. This form can be revealed neither by personnel maintaining the animals nor by veterinary specialists without special tests.

Basing himself on an analysis made in 1871 of an epizootic that broke out in Novoladozhskiy District, St. Petersburg Province, V. F. Nagorskiy pointed out that the disease did not always develop among horses from areas of anthrax danger exhibiting high body temperature. In precisely the same way, in settlements where he analyzed blood, clearly pronounced leukocytosis was discovered among apparently healthy horses without a noticeable increase in temperature; however, the number of such animals falling ill was a minority.

M. S. Gannushkin (1948) indicates that the atypical form of anthrax infection formed encapsulated necrotic foci in the internal organs of animals. This indicates that animals may be latent carriers of bacilli.

The question as to whether or not animals suffering abortive or latent disease could release bacilli into the environment was studied by Kh. Kh. Abdullin, Sh. Kh. Kharisov, D. Sh. Akhmerov, M. N. Levitskaya, M. S. Yezhkova, and Ya. A. Krylov (1971, 1973).

The experiment was performed on 24 milking goats and 13 milking cows. The animals were acquired from anthrax-free farms. The experimental animals had never been vaccinated against this infection. They were infected by a spore suspension of microbes, introduced beneath the mucous membrane of the mouth cavity and the root of the tongue. The infection proceeded as the abortive form in 2 out of 24 infected goats, and it was latent in 5. The disease was acute in 8 out of 13 infected cows; of these, 2 died and 6 recovered; the infection proceeded in the abortive form in 5 animals, without clinically pronounced signs. They could not be distinguished from healthy animals by their behavior. All that was observed was a rise in temperature. The animals were analyzed as possible bacillus carriers and sources. After they were infected, their urine, saliva, and milk were analyzed. The same research was conducted on cattle at three natural anthrax foci. Virulent anthrax bacillus cultures were once against isolated from urine, saliva, and milk from milking cows

exhibiting the clinically pronounced stage of disease and the abortive forms. The bacilli were also detected in the feces and secretions of cows treated with antibiotics and anthrax serum. The microbes were resistant to antibiotics (penicillin, streptomycin). These studies confirm the opinion of some scientists that animals stricken with anthrax may infect the environment.

The anthrax microbe cultures isolated from the bodies of artificially infected cows (reconvalescents) 2 months after infection, and from surviving cows in the natural infection focus 6 months after recovery, were of weakened virulence. They caused the death of subcutaneously infected white mice and, though not always, guinea pigs. In the bodies of the reconvalescents, the pathogen was undergoing deep dissociation; cultures grown on beef-extract agar produced R-, RS-, and S-form colonies. Changes in the morphological and cultural properties of anthrax bacilli in the animal body involving the loss of virulence have also been described by other researchers. D. M. Teternik (1940) isolated six strains of anthrax-like microbes from animals. Of these, two strains reverted into typical anthrax microbe cultures. In a study conducted jointly with Agadzhanov, he isolated a lysogenic apathogenic anthrax strain from the injured intestine of a clinically healthy cow.

In order to determine how the properties of anthrax bacilli change, Kh. Kh. Abdullin (1939) placed typical virulent microbe cultures in collodion bags, which were inserted into the abdominal cavity of rabbits. Periodically (in the course of 90 days) he removed the bags, analyzed their contents, and established change in the morphological, cultural, and biochemical properties and a decrease in virulence of anthrax microbes.

When we examine the epizootic significance of bacillus carriers, we cannot ignore the influence of various predisposing stress factors upon the macroorganisms, under the influence of which the bacillus carrier may begin experiencing an infectious process with the typical clinical signs and pathoanatomical changes. The literature contains indications that animals weakened by various unfavorable conditions are more susceptible to anthrax infection. Feeding is one such factor. According to Opperman, healthy sheep die after being fed 200,000 spores of anthrax pathogen; sheep weakened by starvation, meanwhile, die after being fed just 51,000 spores. Starvation may cause a loss of immunity among animals naturally unsusceptible to anthrax. For example pigeons will fall ill and die when artificially infected with an anthrax pathogen culture following an initial period of starvation.

V. F. Nagorskiy (1902) demonstrated on the basis of a large amount of statistics that weakened animals are the first to fall ill in anthrax-infected settlements.

Complications are observed among such animals following vaccination. A clinically pronounced process may be activated in bacillus carriers by other unfavorable factors as well: chilling, overheating, physical overexertion, and so on. Cases are known in which individual animals have fallen ill in the first days after a lengthy cattle drive and after being transported to slaughterhouses or other delivery points. That they are infected cannot be established while en route. Farms from which such animals come are free of this infection. However, before a final conclusion could be made as to the epizootic significance of carrying and releasing bacilli at times of anthrax infection, deeper analyses employing seroallergenic and bacteriological methods to test suspicious head of cattle would be required.

Infection Transmission Factors

Anthrax pathogen is transmitted to animals and people through meat and animal raw materials (skins, hides, wool, bristles, bones, hair, and so on) obtained from anthrax-stricken animals. Moreover, ill animals infect various objects in the environment. Use of meat from anthrax-stricken animals in feed may cause illness among pigs and various species of carnivorous animals, and in people using such meat for food. Anthrax epizootics may arise owing to contamination of rivers, water basins, and soil by effluents from enterprises processing infected animal raw materials. These transmission factors have special significance to the epidemiology of anthrax infection. M. S. Gannushkin (1948) cites the occurrence of anthrax among soldiers in the Russian Army during the Russo-Japanese War of 1904-1905 resulting from contamination of jackets and headgear by sheepskins (976 cases). He also cited data of American authors indicating that cases of anthrax in 1909-1924 basically involved persons employed in leather industry (60 percent), horse hair and bristle processing industry (10 percent), and wool processing industry (4 percent).

Feed is a significant factor in the transmission of anthrax pathogen. Feed may be infected by spores of this microbe through the most diverse pathways. When hay is harvested from contaminated meadows, microbial spores enter the feed together with soil particles. M. S. Gannushkin (1948) describes the following examples: "Animals maintained at a power mill were fed meal obtained from grain that had been flooded during the high-water season. Three cows died of anthrax. Cases of anthrax ceased to be observed after this meal was removed from the diet of the animals. As a test, the meal was fed to white mice, and an anthrax epidemic began among the latter. It was my duty to certify the death of two horses due to anthrax after feeding on hay from a cart that had previously carried the carcass of a heifer with undiagnosed anthrax subjected to forced slaughter."

Today, at a time of broad development of combined feed industry, special attention should be turned to combined feed as a possible factor of anthrax pathogen transmission. Such feed contains bone, meat, and blood meal, and it is fed to animals of the most diverse species.

The carcasses of animals killed by anthrax are especially dangerous in terms of infection agent transmission. According to research by G. Kebedzhiyev and A.Tomov (1969) and Toschkoff and Veljanov (1970), anthrax infection pathogen does not remain passive in a carcass. In the first hours following an animal's death, microbes present in the carcass begin to intensively reproduce, and their virulence rises. Anthrax microbes in the carcasses of small laboratory animals not only undergo intensive reproduction and raise their virulence, but they also form spores. This information requires further confirmation.

Anthrax bacilli in the stricken organism experience optimum conditions: They exist continuously in vegetative form, and they remain active--they do not form spores. In view of a decrease in oxygen in the body of an animal at the moment of its death, and subsequently in the carcass, complete anaerobiosis sets in, and the bacilli are unable to form spores. In summer, at high temperatures, anaerobes (migrating in copious quantities from the gastrointestinal tract) are the principal form to undergo multiplication in the carcass, many of them being antagonists of anthrax pathogen. As a result of purulent decomposition, the carcass substrate is completely freed of anthrax pathogen. Despite this, however, the anthrax-stricken carcass is not harmless to the environment, since a bloody fluid containing large quantities of vegetative

anthrax bacilli drains from all natural openings of an animal killed by anthrax infection. The bacilli enter the soil together with this fluid, they begin to reproduce at the appropriate optimum conditions, and then they assume their spore form.

When the integrity of the carcass is violated by predatory carnivorous animals and birds, anthrax bacillus spores begin to accumulate within it. The infection spreads from this source by the most diverse pathways (Figure 12).

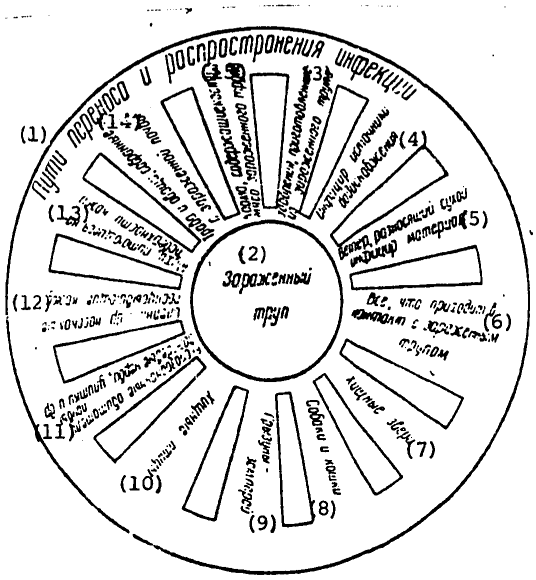

Figure 12. Pathways for Transmission and Spread of Anthrax

Key:

1. Pathways of infection transmission and spread
2. Infected carcass
3. Fertilizer prepared from the contaminated carcass
4. Infected water sources
5. Wind scattering dry infected material
6. Everything coming in contact with the infected carcass
7. Predatory animals
8. Dogs and cats
9. Burrowing rodents
10. Predatory birds
11. Invertebrate soil inhabitants--earthworms, snails, etc.
12. Horseflies and other biting insects
13. Flies feeding on the skin surface
14. Grass and vegetables harvested from contaminated soil
15. Feed containing bone and meat from the contaminated carcass

Invaded soil is an infection reservoir. The concept "reservoir" is not entirely consistent with the nature of anthrax infection in its literal translation--to preserve, to save. Anthrax bacilli present in soil not only survive for a long period of time (more than 70 years), but also under certain conditions they undergo the complete cycle of their development--vegetation and spore formation--many times.

The survivability, reproduction, and variability of *Bac. anthracis* in soil was studied depending on the soil's physicochemical properties and climatic conditions by L. Pasteur (1880), V. F. Nagorskiy (1902), N. A. Pokshishevskiy and A. D. Golovin (1931), A. A. Vladimirov, I. A. Baytin, and A. P. Prokof'yev (1940), K. A. Mirotvorskiy (1940), G. A. Pebsen (1958), R. A. Tsion (1958), A. V. Koronnyy (1958), N. M. Noskov (1958), I. N. Presnov (1956), I. N. Khudaverdiyev, V. L. Cherkasskiy, N. F. Sokolova, E. M. Barsunova, and M. N. Gracheva (1971), A. S. Korotich (1971), G. Ya. Chuyskaya (1971), and others. The most diverse methods were used to study these questions: grazing of animals susceptible to anthrax on the contaminated territory of an animal burial pit; artificial infection of sterilized and unsterilized soil samples by anthrax microbes in laboratory conditions, in cans or in other containers, followed by analysis different times after infection; heating of soil at 75-80° for 15-20 minutes prior to analysis in comparison to unheated soil; placement of microscope slides at different depths in the soil according to the Rossi-Kholodnyy method; landscape experiments; integrated bacteriological and physicochemical analysis of contaminated soil, and comparison of the results with local meteorological data. Other methods for studying epizootic processes are used as well, for example establishing the nature of the infection's spread among animals in a particular territory in connection with the territory's climatic and soil conditions, and in relation to anthrax morbidity computed per 100,000 animals, and the intensity of the epizootic process per 1,000 square kilometers of territory in different soil-climate zones.

Anthrax bacilli can be detected in soil with elective and optimum nutrient media. Fractions of the same soil sample are cultured in the elective medium as an unheated suspension and in the optimum medium following heating at 75° for 30 minutes. The microculture method may be performed with both microscope slides and membrane filters. Among serological methods for anthrax antigen detection, the adsorbed antibody reaction is one used on cultivated soil samples.

The results of these analysis methods may be considered together. Spores and vegetative forms of anthrax pathogen transmitted into soil together with carcasses or the excretions of sick animals may survive within the soil under optimum conditions without losing their pathogenicity for decades. The following make up the optimum conditions permitting anthrax microbe to undergo its developmental cycle many times--that is, germination of spores into vegetative forms, reversion to the spore state following a certain period of multiplication, and so on: a sufficient quantity of organic substances (humus content 4 percent and higher), soil moisture content 60-80 percent of total moisture capacity, pH close to neutral or weakly alkaline, temperature not lower than 16-18°, satisfactory aeration. Such conditions are satisfied by the soil of irrigated meadows, marshy, peaty, chernozem, and alluvial river basins, dry river channels, and dry lake beds. Spores may germinate in gray podzolic acid soils with a humus content of 1.83 percent, but subsequently their growth ceases, and vegetative forms die off. Spore germination has not been revealed in ordinary sand not containing humus. Anthrax bacilli buried in clay with uncut carcasses and in the form of liquid spore culture undergo inactivation rapidly.

In the period before vaccination was introduced, anthrax microbes were able to survive in soil with physicochemical properties unfavorable to their existence only owing to annual replenishment of the population through the discharges of sick animals and the carcasses of animals killed by anthrax. In the era following vaccination, after animals began to be subjected to vaccination, the microbe may have undergone self-sterilization in regions containing the soils indicated above, since entry of microbes into the soil decreased dramatically. For example in former times the largest number of anthrax cases was recorded in the country's northwestern regions, where the soil pH was 6.5 and higher. Anthrax has been encountered extremely rarely since 1945 in the eastern and southern regions, or it has not been observed at all; soil pH in these areas was 5.5 and lower. Even more indicative are the dynamics of anthrax epizootics in the Yamalo-Nenets National Area, where 66 anthrax epizootics were recorded from 1898 to 1941, causing the death of over 1 million reindeer. Since 1941, not a single outbreak of anthrax has been recorded on this territory among either reindeer or people; the soil pH is 3.5 and lower, and the soil is deficient in organic substances and microelements.

Soil infected by anthrax pathogen is encountered over our entire planet, but it is focal in nature--that is, its occurrence is limited to particular territories. A. A. Vladimirov and I. A. Baytin (1940) write that an expedition of the Leningrad Veterinary Scientific Research Institute studied a large number of animal burial grounds, where it was known that the carcasses of anthrax-stricken animals had been buried at times. The research was conducted along the entire course of the canals of the Mari system, from Shlissel'burg to Novaya Ladoga and from Vytegra to Chayka, known in the past for its anthrax danger. The authors point out that anthrax spores could be detected only in some burial grounds located in humic or peaty ground and, moreover, many years (up to 30) after the last burial of anthrax-stricken carcasses. On the other hand despite numerous meticulous analyses, anthrax spores were never detected in animal burial grounds in clayey soil, irrespective of the time of the last burial and the extent of carcass decomposition.

According to K. A. Mirotvorskiy (1940) anthrax soil foci are also irregularly scattered over the territory of the Murgab Plain in the Turkmen SSR. The distribution of the foci depends on variations in the salt composition of different soil areas. Saline and salinized soils are encountered there in the form of numerous and diverse depositions and small areas, which restrict the possibilities for not only vegetation but also survival of anthrax microbes. Soils having physicochemical properties favoring the existence and development of anthrax microbes may experience the unfavorable influence of biological factors of the ecosystem. Such factors include: general soil toxicity, which has an inhibitory effect upon not only pathogenic microbes but also the microflora of the soil itself; the antagonistic influence of soil microflora.

Anthrax Microbe Antagonists in Soil

The species composition of soil microorganisms depends not only on the physicochemical properties of the soil but also on the species composition of cultivated plants; the latter form the rhizosphere, certain representatives of which may have antagonistic action upon anthrax pathogen. N. A. Krasil'nikov was able to isolate a tremendous quantity of strains of bacteria and actinomycetes--antagonists of pathogenic microbes--from soil.

V. V. Arkhipov (1951, 1954) established that clover, rhubarb, vetch, wheat, rye, and garlic have an inhibitory action upon anthrax bacilli present in soil. Similar data were obtained by V. S. Zarubkinskiy (1953) with cultivated garlic, onion, rye, wheat, barley, clover, alfalfa, and vetch.

A. S. Korotich, L. D. Kalyuzhnaya, and G. Ya. Chuyskaya (1969) and L. I. Pogrebnyak (1974) established that the total content of actinomycetes was higher in the soil of animal burial grounds than in soil from control samples. Soil from animal burial grounds also contained more actinomycetes with antagonistic properties than in the control (72 percent as opposed to 64 percent). Strains of actinomycetes exhibiting more-pronounced antagonistic activity were isolated from soil samples taken from animal burial grounds in chernozem soil. No differences were established between virulent and vaccine anthrax microbe strains and *Bac. cereus* in terms of sensitivity to the antagonistic action of actinomycetes. The antagonism of isolated antinomycete strains was studied in solid and liquid media. Culture fluid containing especially active actinomycete strains inhibited growth of anthrax microbes at a 1:400 dilution. A. S. Korotich et al. tested two of the more-active actinomycete strains in experiments having the purpose of eradicating anthrax microbes from soil. These strains were introduced into chernozem soil with a high humus content, and they had a bactericidal action upon anthrax pathogen.

It might be suggested that these data might be used as a basis for studying procedures for removing anthrax agents from soil by the introduction of antagonist biomass. However, simple introduction of the antagonist into soil may not produce the desired result. Competition for nutrients may arise between the introduced antagonist and the soil's native microflora. Bactericidal substances excreted by the antagonist may be decomposed quickly by the microflora. Therefore after antagonists are discovered and after they are obtained in culture, we would have to learn how to create favorable ecological conditions for them in soil.

Bacteriophages seem to be another biological factor having a certain influence upon anthrax bacilli in soil. Vegetative forms of anthrax pathogen undergo lysis in response to the action of virulent phage, while moderated phage may elicit lysogen formation, which would cause change in the properties of the microbe. V. L. Larina and L. Ye. Petrova (1964) isolated active phage from lysogenic cultures of anthrax bacillus isolated from soil sampled from the burial sites of the carcasses of animals killed by anthrax.

The Anthrax Focus Concept

The soil focus or unfavorable point* is the most stable element of the epizootic process of anthrax. The geographic size of an anthrax focus may be quite diverse--from one of insignificant size (a place where carcasses of animals killed by anthrax are buried) to the vast territories of cattle drive routes, particular pastures, or even the pasturelands of almost an entire country (Turkey, Iran). While soil may be infected by anthrax pathogen uniformly and over a long period of time, stable foci of this infection form only in particular limited territories.

[* Translator's note: The term "unfavorable point", a direct translation of "neblagopoluchnyy punkt", will be used subsequently in lieu of a satisfactory English equivalent. See next paragraph for the author's definition.]

For practical purposes an anthrax soil focus reveals itself through infection, within its territory, of agricultural animals or, very rarely, man--that is, only after the epizootic cycle is closed. It is rather difficult to establish the boundaries of a focus. B. L. Cherkasskiy and K. M. Kamenetskiy (1971) suggest treating a soil focus of anthrax infection as a place where an animal infection had arisen, and all of the surrounding territory within which farm animals had grazed. N. Zhanuzakov (1970) does not treat the concepts "focus" and "unfavorable point" as being synonymous; he suggests using the term "unfavorable point" in application to a farm or settlement within the territory of which cases of anthrax and deaths due to anthrax have been noted among farm animals, and the term "infection foci" in application to individual points within the former. N. A. Shchelokov (1971) agrees completely with this definition; he writes: "In my opinion it would be correct to interpret a separate population point, a tract, a pasture, or a separate grazing area as an anthrax-unfavorable point, and a place of burial of an anthrax-stricken carcass, a place of slaughter of an anthrax-stricken animal, and a separate yard or animal building in which an animal had contracted anthrax and was killed by it as an anthrax focus."

The activity of an epizootic focus depends on the activity of the soil focus and on the number of susceptible animals present. A. S. Korotich (1969) classifies anthrax foci as follows:

Active, mildly active, and inactive. Active foci are ones in which anthrax outbreaks are recorded among animals every year or within short intervals;

mildly active foci are ones in which anthrax outbreaks occur less frequently and their periodicity is lower than in active points;

inactive foci are ones in which one outbreak is recorded every 10 years.

Pasteur (1880) proved in his time that earthworms cause vertical movement of anthrax pathogen in soil. This phenomenon was subsequently confirmed by other researchers. Other invertebrate soil inhabitants--snails and bugs--and burrowing rodents can also carry the pathogen from underlying layers to the surface.

A. F. Nadzhafov (1971) established on the basis of his own observations that a certain correlation exists between the abundance of field rodents in anthrax foci and infection of agricultural animals: the higher the abundance of rodents, the more disease agent spores are carried to the soil surface and the higher the probability of infection of agricultural animals.

Other natural factors as well as human activities disturbing the physical structure of the land also play a significant role in movement of anthrax microbes from deep soil and in expansion of the invaded territory. Such factors include river floodwaters that erode the soil. Bluchmann (1962) described a case of infection of cattle on a pasture where the last outbreak of disease had been recorded 70 years previously. He explains this by erosion of the soil of the grazing area by runoff.

V. I. Adamovich and N. N. Nikonov (1970) indicate a direct dependence between the quantitative distribution of anthrax foci and the intensity of land erosion: The more intense the erosive processes are, the more anthrax foci arise.

In arid seasons of the year anthrax agent spores may be carried rather long distances by dust storms. Intensification of excavation operations is acquiring a certain amount of significance today as a cause of reactivation of dormant anthrax foci.

M. G. Kondakov (1969) reports that an anthrax outbreak occurred at a dairy farm after a trench 2.5 meters deep was dug. It turned out that the sovkhoz farm was located on the territory of a former animal burial ground. Animals killed by anthrax had been buried there 50 years ago. There was once a case in which anthrax arose 76 years following the last case of illness owing to well drilling and blasting of soil on the territory of a former burial ground (N. M. Noskov, 1965). V. I. Karyagin (1971) states that anthrax infection is encountered more frequently in herds grazing near areas of reclamation and other excavation operations, and in river channels with eroded banks, and in winter among animals fed hay procured from plots located not far from the sites of excavation operations. K. R. Urguyev, P. I. Golin, and I. M. Belan (1971) note that in former times, anthrax was encountered among animals in the Dagestan ASSR usually in mountainous terrain, while in more-recent years, especially after 1965, cases of anthrax have been observed mainly on the plains. They explain this phenomenon by the increased volume of land management and hydraulic engineering operations, which are being conducted without a consideration for the anthrax hazard offered by the land.

It follows from this that we must be mindful of possible anthrax outbreaks among animals near areas of excavation operations, if the animals had not been previously subjected to vaccination. Therefore before excavation operations begin, the anthrax infection hazard offered by the territory of future excavation operations must be clarified. For this to be possible, all old and newly revealed anthrax foci need to be meticulously recorded.

Anthrax microbes present in soil may undergo change. Their pathogenicity may weaken or disappear entirely in response to unfavorable conditions or as a result of a prolonged saprophytic way of life. This phenomenon causes change in the activity of the anthrax soil focus.

Water is a significant factor involved in the transmission of anthrax pathogen to susceptible animals. According to R. A. Tsion (1930), G. P. Rudnev (1950), A. A. Koronnyy (1955), and A. D. Garmazova and M. A. Konstantinova (1961) anthrax bacillus may survive up to 10 years in water as spores. The spores may be introduced into water basins by spring floodwaters. Given a sufficient concentration of blood in water, anthrax agents may proliferate in capsule form (Kh. Kh. Abdullin, T. V. Kaparovich, 1973).

The Means of Infection Transmission, and the Ways of Its Spread

Cases of infection of a healthy animal via direct contact with an anthrax-stricken animal have not been described in the literature. The infection agent is transmitted only through contaminated objects. The pathogen is transmitted from the body of a sick animal to the environment together with feces, urine, and milk. The pathogen may be eliminated from the body of an animal exhibiting clearly pronounced clinical disease and from an animal exhibiting a diffuse clinical pattern of disease. When such animals are present in a building, these excretions come in contact with various animal care objects, as well as with feed and water.

M. S. Gannushkin (1948) cites the following examples of this infection pathway: "Fresh scratches were discovered on the skin of an anthrax-infected horse at the place of contact with the harness. These scratches served as an avenue of anthrax infection, since not long prior to this a collar removed from another horse that had died of anthrax was placed on this horse. The first death of an animal due to anthrax at the farm was followed by another three cases of illness. Arisal of the latter is explained by the fact that the person caring for the animals cleaned the feed boxes with a broom that had been highly contaminated by blood from a bull killed prior to this by anthrax."

Animals may also be infected by the alimentary pathway when grass contaminated by soil particles and feed infested by spores of this microbe are eaten. This infection transmission factor is acquiring extremely important significance with creation of larger farms. Special attention must be turned to the use of combined feeds, which contain a large number of components of both plant and animal origin. The foreign literature describes many cases of infection of animals by anthrax through infected feed containing meat and bone meal.

According to Spears and Davidson (1959) 4,104 cases (88 percent) of anthrax contracted by 4,668 animals (cattle and pigs) in England in 1952-1958 were the result of eating infected feed imported from other countries. It is dangerous to feed the meat of anthrax-stricken animals in fur farms.

Anthrax infection may also occur when the pathogen enters the respiratory tract. M. S. Gannushkin writes that this infection pathway apparently plays an insignificant role in relation to cattle. Animals of other species may also be infected by spores inhaled with dust. Gannushkin cites the following example. Anthrax broke out in one flock of sheep under the following conditions. The sheep were regularly driven to pasture on a particular road. This road was improved by covering it with gravel from a pit in which the carcasses of animals killed by anthrax had previously been buried. As they moved along, the sheep raised dust, and inhaling it, they infected themselves with anthrax. Analysis of the gravel revealed a large quantity of anthrax spores.

Insects, birds, rodents, and some species of unsusceptible animals play a large role in the transmission of anthrax pathogen.

The significance of blood-sucking insects as a transmission factor in the epizootiology of anthrax infection was studied by V. F. Nagorskiy (1902), S. N. Vyshelesskiy and S. I. Rasputin (1916), N. G. Olsuf'yev and P. P. Lelep (1935), P. P. Lelep (1936), and others. They believe that horseflies play the leading role in this means of infection spread. That these insects are capable of mechanically transmitting infection from a sick to a healthy animal was proven in experiments on laboratory and agricultural animals. N. G. Olsuf'yev and P. P. Lelep established survival of the pathogen in the oral apparatus of a horsefly for up to 7 days, in the craw for up to 1 day, and in the gastrointestinal tract for up to 7 days. Horseflies eliminate anthrax pathogen with feces for a period of 5-9 days. Attempting to suck uncoagulated blood from a fresh carcass, a horsefly infects its proboscis (Figure 13).

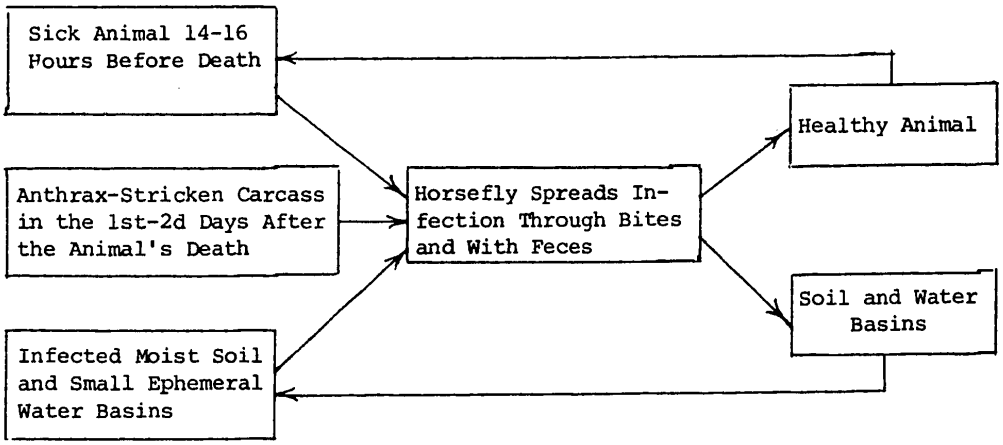

Figure 13. Spread of Anthrax by Horseflies

In addition to horseflies, fireflies and mosquitoes may be involved in mechanical transmission of anthrax from a sick animal or a fresh carcass. However, these insects have less significance than horseflies in development of anthrax infection.

The literature contains reports of transmission of anthrax pathogen by ticks from a sick to a healthy animal, and expansion of infected territory by them. A. G. Gadzhiyev, F. A. Abushev, and S. I. Yuditskaya (1965) allowed Haemasid ticks to feed on rodents artificially infected by anthrax agents. Anthrax culture was isolated from the bodies of ticks removed from infected Persian gerbils and voles. In one case the infection was transmitted by infested ticks to a healthy gerbil.

Ananthapadmanabham (1962) isolated anthrax pathogen from several species of ticks removed from the carcasses of sheep killed by this disease. Bacilli were not isolated by the culturing method from ticks of the same species removed from a healthy sheep, but a biological test confirmed presence of infection. The author believes it an established fact that ticks carry *Bac. anthracis*. O. V. Zdorik (1955) infected carcass beetles of several species by allowing them to feed on the carcasses of laboratory animals dying following artificial infection by anthrax, or animals doused in a culture of this microbe, and he analyzed the intestines of the beetles and their carcasses. Anthrax pathogen was discovered in the intestine of the burying beetle and the rove beetle, analyzed immediately after feeding on carcasses. P. P. Stepaykin (1937) studied the susceptibility of gray rats to anthrax. He observed deaths among these rodents following their feeding upon the carcasses of experimental animals killed by anthrax. An anthrax pathogen culture was isolated from the carcass of a fallen rat. Five out of six gray rats infected with this isolated culture subcutaneously or by the alimentary pathway died. Experiments were conducted in which gray rats were artificially infected by a laboratory strain of anthrax pathogen, which turned out to be less virulent than the rat-borne strain.

Performing similar studies, G. S. Zelenenko (1956) also established a certain resistance of gray rats to artificial subcutaneous and enteral infection by anthrax material. Nungester, Moore, Mika, and Summers (1955) concluded from an experiment that rats are more resistant to anthrax than are guinea pigs. Taylor, Kennedy, and Bundell (1961) report rapid decline in sensitivity of animals to anthrax microbe with increasing age. With each subsequent day, the LD_{50} increased by 50 percent. The same authors (1963) were unable to establish this law for rats raised in special sterile conditions.

N. A. Mikhin (1942) notes spontaneous infection of gray rats by anthrax.

Consequently it may be suggested that rats do not possess natural inborn immunity to anthrax infection. Immunity is apparently acquired during the life of the animal as a result of cross-immunization by particular representatives of the body's rich natural microflora possessing antigens in common with the anthrax pathogen.

Ye. Ye. Punskiy and F. S. Tsebulevskaya (1958) demonstrated the possibility of anthrax infection among large gerbils inhabiting territories favoring this infection. In April 1957 an anthrax pathogen culture was isolated from eight large gerbils by the biological assay method in Chardzhouskaya Oblast, Turkmen SSR, on the bank of the Amu-Dar'ya River. These rodents were infected by an anthrax pathogen strain isolated in 1956 from the skin of a slaughtered sick camel. The gerbils died in response to subcutaneous injection of 2 billion microbial cells. When the same quantity of bacteria was introduced *per os*, some of the gerbils survived. The experiment was repeated on animals with an injured oral mucous membrane. All gerbils died.

The authors conclude that infection of large gerbils in nature by the alimentary pathway is possible only if the mucous membrane of the oral cavity and the gastrointestinal tract is injured. Yu. S. Voronin, S. A. Dzharylgasov, Yu. S. Pisarevskiy, and M. M. Faybich (1963) and M. A. Musayev, F. A. Abushev, and S. Kh. Yuditskaya (1966) also established high sensitivity of large gerbils to experimental anthrax infection. A. G. Korol' (1962) noted in studies of the participation of mouse-like rodents in the anthrax infection cycle that the anthrax pathogen undergoes lysis and degeneration in the body of the field mouse and the gray hamster, and that this is what possibly keeps the rodents from dying. That susliks are infected by anthrax in nature is indicated by V. M. Tumanskiy (1950) and by G. G. Baktygaliyev, N. P. Limanskiy, A. F. Lavrent'yev, S. I. Ivanov, A. A. Altukhov, L. V. Filimova, V. K. Volobuyev, Ye. I. Rybak, E. F. Gorbunova, and K. T. Bobyshbayev (1972).

The fact that anthrax pathogen has been isolated from the bodies of trapped clinically healthy wild rodents is highly interesting. The significance of such animals to the epizootiology of anthrax has not been clarified.

It should be kept in mind in experiments concerned with the susceptibility of wild animals to anthrax that caged maintenance in captivity is a highly intense stress factor to them. Many of them die without any sort of infection under such conditions. This question deserves organization of special experiments in natural field conditions. The study area should be dependably screened off in order to prevent exit of experimental animals and entry of other animals and insects. The experimental animals should be infected only by permitting them to feed on carcass material.

In order to make the conditions more natural, insects and arthropods may be released into this isolated space (isolated, it would be presumed, by the appropriate metallic netting).

Special experiments were conducted in order to clarify the role of birds in the epizootiology of anthrax. I. V. Popov (1926) used the carcasses of white mice killed by anthrax as a result of artificial infection to feed experimental birds. In order to obtain spore-containing materials the carcasses of the mice were dismembered and placed in a thermostat for 1 day, after which they were fed to the birds. The experiment was performed on two hawks, one kite, one steppe harrier, two red-footed falcons, one raven, three hooded crows, two rooks, and four jackdaws. The hawks, the kite, and the steppe-harrier had to be force-fed.

All feces were collected daily and subjected to bacteriological analysis from the moment feeding started. The appropriate dilutions of fecal suspensions were heated at 60-65° for 30-45 minutes prior to being seeded on agar in Petri dishes. It was established by the experiment that beginning with the second day after feeding started, the birds began to eliminate anthrax pathogen spores together with feces. One loop of isolated agar culture suspended in 5 ml physiological table salt solution caused the death of mice in the first day after infection by a dose of 0.01 ml.

V. Kachakhidze (1926) fed anthrax-containing material to an eagle irregularly in the course of a year. Feces were studied by I. V. Popov's method, and anthrax agent cultures virulent in relation to rabbits were always isolated.

G. V. Kolonin (1972) reports that he also was able to discover anthrax spores in the feces of birds fed infected meat. Necrophagous griffins have special significance to mechanical transmission of anthrax pathogen.

Veterinarian V. P. Slentsov reports sensitivity of swallows to anthrax, which he observed during an anthrax outbreak among deer in 1969-1970 in the Yakut ASSR.

Seasonality and Natural-Geographic Distribution of Anthrax

Anthrax epizootics of the prevaccination period, which was typified by universal pasture maintenance of farm animals, were characterized by clearly pronounced seasonality resulting from two factors: the pathogen's activity in the principal reservoir--the soil, and presence of insects--infection vectors. The soil, climatic, and topographic conditions responsible for activating these factors differ in different geographic zones. In steppe regions the soil conditions favored survival and activation of the disease agent, and infection occurred on pasturelands by the alimentary pathway. The dynamics of epizootic development could be described by a flattened curve. The first sick animals appeared in January, in subsequent months the number of stricken animals gradually increased, and it attained a maximum in June-August. An especially steep increase was observed in the dry period of the year, when the probability of animal infection increased due to dessication of grass.

In the north, in the taiga and tundra, anthrax pathogen could be detected in soil only because its numbers were constantly replenished together with excretions from sick animals and with carcasses. The spread of epizootics, meanwhile, was the product of the transmissive infection transfer mechanism. Major epizootics arose

in July and at the beginning of August, which coincided with mass migrations of vectors--horseflies and houseflies. After insect activity subsided (in response to colder weather), the number of cases of disease decreased dramatically.

Mass vaccination of animals against anthrax and restriction of pasture maintenance promoted change in the epizootic process of this infection--in its seasonality, the intensity of its course, and its total elimination from certain territories.

According to S. I. Dzhupina (1973) the incidence of anthrax among animals decreased so much in 1961-1970 in the European SSR between 60 and 65° North Latitude that it became impossible to establish a seasonal law. Seasonal manifestation of the disease hardly changed at all in the remaining territory of this zone, south of the indicated latitude. The author suggests on the basis of the fact that seasonality is evident even with mass immunization of animals that in nature, anthrax pathogen constantly circulates among animals of certain species. In particular, rodents may be such animals.

Today, in addition to natural conditions, the methods of animal husbandry also have an effect on the seasonality of anthrax infection; they may also predetermine the pathways of animal infection. P. N. Burgasov, B. L. Cherkasskiy, A. M. Marchuk, and Yu. F. Shcherbak (1970) cite the following as anthrax seasons in different countries: Denmark, FRG, GDR--January-March; Switzerland, Argentina--February-March; Portugal, Czechoslovakia--May; Soviet Union, Hungary, Romania, Spain, Turkey, India--June-August; Bulgaria, France, Poland, Iran, Yugoslavia, Iraq, Morocco--August-September; Belgium, Great Britain, The Netherlands, Australia--October-December. Anthrax outbreaks occur in winter in Denmark, the FRG, the GDR, Switzerland, Belgium, The Netherlands, and Great Britain owing to extreme limitation of pasture maintenance of farm animals, and abundant use of imported combined feeds. In all other listed countries the outbreaks of this infection are observed in summer (February and December are summer months in Australia and Argentina), which is the product of preferential maintenance of animals on pastures.

The literature contains information indicating that increases in incidence of anthrax among agricultural animals are noted after particular time intervals; such recurrence of epizootics over time intervals measured in years is a consequence of the periodicity of epizootics. M. S. Gannushkin (1948) states that devastating epizootics were observed among deer in 1877, 1897, and 1907 in Arctic tundra in the former Arkhangel'sk Province. According to N. A. Mikhin (1942) the years 1890, 1898, and 1901 were especially unfavorable in relation to incidence of anthrax among agricultural animals, while 1921 was a bad year in the RFSFR.

The periodicity of all epizootics can be explained by natural immunization of susceptible animals during an epizootic, which leads to the latter's extinction. Genetic variability of the components of the epizootic process--the parasite and the host--plays an important role in acquisition of immunity by susceptible animals in an epizootic focus. Populations of pathogen in an epizootic focus may vary in their virulence. Animals infected by a weakly virulent pathogen may acquire immunity, or they may survive the disease without displaying clinical symptoms. Individuals exhibiting greater natural resistance to infection may be present among animals. Their infection by a highly virulent pathogen may result in latent infection and formation of immunity. Animals that had suffered disease with a typical clinical pattern also develop immunity.

This law is also inherent to the anthrax epizootic process, but it has not been studied well. For example, we have not revealed the time recovered animals remain immune. In the past, the dramatic decline in numbers of animals, caused by their mass death, influenced the extinction of major epizootics. The nature of epizootics in taiga regions depended on fluctuations in the abundance of insect vectors. Such periodicity was not observed in the steppe provinces of Russia. Outbreaks of anthrax epizootics appeared from year to year, with insignificant variations in the degree of their manifestation. In certain years increases in the numbers of sick animals coincided with droughts. Science is presently unable to make any conclusive judgments as to the periodicity of anthrax epizootics among agriculture animals owing to immunization in the course of the epizootic process.

The ecological conditions of different geographic zones impose unique features upon the way any epizootic process, including anthrax, manifests itself. These conditions influence the physiological state of the organism, the range of the vectors, their activity, the seasonality with which epizootic and enzootic outbreaks arise, and so on. Classification of individual epizootics in relation to different landscapes has been proceeding actively in recent years. Analyzing the factors governing arisal, development, and extinction of anthrax epizootics in the prevaccination period, G. V. Kolonin (1970) developed a landscape classification of anthrax foci.

The capability of anthrax pathogen spores for surviving dozens of years in certain types of soils without losing their virulence, their capability for infecting animals by alimentary and transmissive pathways, their ability to infect animals of many species, and cases of isolation of the microbes from the bodies of wild animals and rodents led some researchers to classify anthrax as one of the natural-focal diseases. Professor I. G. Galuzo defines natural focal occurrence of diseases as deposition of infectious disease foci in nature and their existence independently of man and agricultural animals.

Anthrax is a soil infection. Its causative agent is not one of the full-fledged components of the life cycle of the soil ecosystem. It survives in soil mainly in spore form, and prolonged vegetation in this substrate leads to change in its properties. Epizootic outbreaks of this infection are observed today mainly as a result of disturbance of the soil cover of old infection foci.

Cases of infection of mouse-like rodents by anthrax pathogen deserve great attention (V. N. Lebedev and V. M. Strel'cheva, 1969; B. L. Cherkasskiy and L. M. Marchuk, 1971). The disease agent can be isolated from the bodies of entirely healthy animals, and dissection of slaughtered animals fails to reveal pathoanatomical alterations in the organs typical of anthrax. The isolated anthrax culture is weakly virulent or completely avirulent and unencapsulated. Transmission of infection from wild to domestic animals is extremely rare, if it ever does occur. On the contrary wild animals are infected by foci created through infection of domesticated animals. All described enzootics of anthrax among wild animals, including the enzootic of Kruger Park in the UAR (Pienaar, 1967) and among forest-dwelling bison in Canada (Cousineau, Clenagham, 1965), arose as a result of penetration of infection from anthrax foci of agricultural animals.

Infection of wild animals, especially ungulates, may be the reason for the persistence of anthrax foci. V. L. Adamovich (1972) notes that anthrax foci in

forested terrain of Bryanskaya Oblast are persistent wherever wild ungulates come into contact with pastures used by domesticated animals. Diseases exhibiting a natural focal nature are limited to a certain geographic territory, to an ecosystem inherent to such a territory, one promoting reproduction and circulation of the pathogen on certain members of this ecosystem. Anthrax is a global infection, and its local occurrence in particular territories is the product of physicochemical features of the soil.

BIBLIOGRAPHY

1. Abdullin, Kh. Kh., Kharisov, Sh. Kh., and Akhmerov, D. Sh., "Dostizheniya nauki i praktiki v oblasti mikrobiologii i epizootologii" [Scientific and Practical Achievements in Microbiology and Epizootiology], Tartu, 1973, p 114.

2. Adamovich, V. L., and Nikonov, N. N., ZHMEI, Moscow, No 6, 1970, p 73.

3. Arkhipov, V. V., TRUDY GNKI, Moscow, No 17, 1971, p 248.

4. Akhundova, K. A., ZHMEI, Moscow, No 10, 1967, p 129.

5. Bakulov, I. A., and Tarshis, M. G., "Geografiya bolezney zhivotnykh zarubezhnykh stran" [Geography of Animal Diseases of Foreign Countries], Moscow, 1971.

6. Baroyan, O. V., "Ocherki po mirovomu rasprostraneniyu vazhneyshikh zaraznykh bolezney cheloveka" [Notes on Worldwide Occurrence of the Most Important Human Infectious Diseases], Moscow, 1967.

7. Burgasov, N. N., Cherkasskiy, B. L., Marchuk, A. M., and Shcherbak, Yu. F., "Sibirskaya yazva" [Anthrax], Moscow, 1970.

8. Vladimirov, A. A., and Baytin, L.A., "Sibirskaya yazva. Raboty XI plenuma veterinarnoy sektsii VASKhNIL" [Anthrax. Proceedings of the 11th Plenum of the Veterinary Section of the All-Union Academy of Agricultural Sciences imeni V. I. Lenin], 1940.

9. Vyshelesskiy, S. N., and Rasputin, S. I., AVN, No 5, 1916, p 581.

10. Gannushkin, M. S., "Obshchaya epizootologiya" [General Epizootiology], 1948.

11. Dzhupina, S. I., "Dostizheniya nauki i praktiki v oblasti mikrobiologii i epizootologii," Tartu, 1973, p 52.

12. Zhanuzakov, N., VETERINARIYA, No 5, 1970, p 66.

13. Karyagin, V. I., "Aktual'nyye voprosy profilaktiki sibirskoy yazvy v SSSR" [Pressing Problems in Anthrax Prevention in the USSR], Moscow, 1971, p 43..

14. Korotich, A. S., Kalyuzhnaya, L. D., and Chuyskaya, G. Ya., SBORNIK "ZDOROV'YE", Kiev, No 3, 1963, p 7.

15. Kraminskiy, V. A., and Sorkin, Yu. I., "Problemy osobo opasnykh infektsiy" [Problems of Especially Dangerous Infections], Moscow, Vol I, 1970.

16. Mirotvorskiy, K. A., "Sibirskaya yazva. Raboty XI plenuma VASKhNIL" [Anthrax. Proceedings of the 11th VASKhNIL Plenum], Moscow, 1940, p 38.

17. Mikhin, N. A., "Sibirskaya yazva u cheloveka i s.-kh. zhivotnykh" [Anthrax in Man and Agricultural Animals], Moscow, 1942.

18. Noskov, N. M., "Sbornik nauchnykh trudov Estonskoy s.-kh. akademii" [Collection of Scientific Works of the Estonian Agricultural Academy], Tartu, Vol 4, 1958, p 69.

19. Olsuf'yev, N. G., and Lelep, P. P., "Parazity perenoschiki i yadovityye zhivotnyye" [Parasitic Vectors and Poisonous Animals], Moscow, 1935.

20. Pebsen, E., "Sbornik nauchnykh trudov Estonskoy s.-kh. akademii," Tartu, 1958, p 4.

21. Pogrebnyak, L. I., VETERINARIYA, Moscow, 1973, p 6.

22. Romanov, G. I., TRUDY GNKI, Moscow, Vol 16, 1969, p 204.

23. Romanov, G. I., TRUDY GNKI, Moscow, Vol 17, 1971, p 234.

24. Urguyev, K. R., Golin, P. I., and Belan, I. M., "Aktual'nyye voprosy profilaktiki sibirskoy yazvy v SSSR," Moscow, 1971, p 51.

25. Tsion, R. A., "Sbornik nauchnykh trudov Estonskoy s.-kh. akademii," Moscow, 1958.

26. Cherkasskiy, B. L., and Kamenetskiy, K. M., "Aktual'nyye voprosy profilaktiki sibirskoy yazvy v SSSR," Moscow, 1971, p 70.

27. Chuyskaya, G. Ya., "Aktual'nyye vorposy profilaktiki sibirskoy yazvy v SSSR," Moscow, 1971, p 72.

28. Shchelokov, N. A., "Aktual'nyye vorposy profilaktiki sibirskoy yazvy v SSSR," Moscow, 1971, p 27.

29. Brown, E., J. BACTERIOL., Vol 69, No 5, 1955, p 590.

30. Druett, H., Henderson, D., Packman, Z., and Peacock, S., HYG. J., Vol 51, No 3, 1953, p 359.

31. Gordon, M., and Barton, B., ARCH. INDUSTR. HYG. AND OCCUPAT. MED., No 10, Sec. 1, 1954, p 16.

32. Nordberg, V. K., VETERINARMED., No 5, 1953, p 915.

33. Nungester, W., Moore, V., Mika, L., and Summers, P., J. INFECT. DISEASES, Vol 96, No 3, 1955, p 227.

34. Smith, H., and Keppie, J., BRIT. J. EXPTL. PATHOL., Vol 36, No 5, 1955, p 460.

35. Spears, H., and Davidson, J., VET. REC., Vol 71, No 31, 1959, p 637.

DIAGNOSIS

There are now a significant number of sophisticated methods for diagnosing anthrax. These include bacteriophagy, the luminescent-serological method, the "necklace" reaction, quick diagnostic methods revealing the capsulogenic properties of anthrax pathogen in artificial nutrient media and in the bodies of laboratory animals, and so on. In addition to the latest methods, the methods of bacterioscopic, microbiological, serological, and biological diagnosis are extensively employed.

An anthrax diagnosis is made on the basis of epizootic data describing the disease symptoms and the results of laboratory analysis of pathological material.

Clinical-Epizootiological Diagnosis

Epizootic indicators reflect cases of anthrax among animals, occurring formerly or observed presently in a particular place, and the epizootic situation in surrounding farms. These data are used to determine the need for appropriate measures.

Animal anthrax is defined clinically in terms of the disease course, which may be fulminant, acute, subacute, and chronic.

Depending on the means of infection, we distinguish between the dermal (carbuncular) form of disease, which develops when the pathogen penetrates through the skin as a result of bites by biting insects or through wounds and scratches, and the intestinal form, in which the pathogen enters the body with feed or water. This division is conditional, since intestinal affliction may be observed in the presence of the dermal form, and inflammatory edema may be observed on the skin and in organs with the intestinal form. Anthrax usually proceeds acutely. The incubation period is short--1-2 days. With an acute course the temperature of the animals rises to 41-42°, pulse and respiration rise, breathing becomes labored, the animals are extremely subdued, and sometimes they are highly excited due to stimulation of the central nervous system. Appetite is absent, and rumination ceases in cattle. Mucous membranes of the nasal and oral cavities are cyanotic. The animals move about with difficulty, and they lie down frequently. Gastrointestinal disorders are observed with the intestinal form of anthrax, while horses reveal signs of cholic accompanied by elimination of bloody fluids.

The carbuncular form of anthrax usually occurs among horses and cattle; in this case it may be primary, appearing at the place of introduction of the pathogen by the bites of blood-sucking insects, or secondary, taking the form of edema and

infiltrates on the head, chest, and shoulders, and in the area of the abdomen, the submaxillary region, the (vymen'), and the scrotom.

At the beginning of disease, edematous areas are hot, hard, and painful, and later they become cold and painless. The carbuncular form of anthrax arises in animals grazing in forested areas containing many biting insects.

Anthrax may be chronic in cattle. In small ruminants it usually has a fulminant course, terminating with the animal's sudden death. Anthrax proceeds in pigs usually as a localized process taking the form of angina or pharyngitis, chronically for the most part, and sometimes subacutely or acutely.

It is impossible to make a diagnosis only on the basis of epizootic and clinical data. The latter indicate only a need for implementing therapeutic, preventive, and sanitary measures. A conclusive diagnosis must be made on the basis of a laboratory analysis of pathological material from the carcass of a fallen animal.

As we can see from the above, it is presently impossible to make a conclusive and accurate diagnosis of anthrax while the animal is alive, since specific analysis methods do not exist. Thus veterinary scientific research institutions are faced by the task of developing methods for diagnosing anthrax while the animal is still alive (allergic methods as an example), and for revealing sick animals in areas of chronic disease and in the presence of unfavorable epizootic situations.

Clinical-Epizootic Differential Diagnosis

An anthrax diagnosis must rule out certain other infectious animal diseases, for example cattle and sheep pasteurellosis, cattle emphysematous carbuncles, sheep diseases elicited by the causative agents of the malignant edema group, and piroplasmosis.

The clinical signs of acute pasteurellosis have some similarity with symptoms observed in the presence of anthrax; however, pasteurellosis is a disease of large and small horned animals and pigs. Moreover anthrax can cause the death of horses and other animals, while among pigs this disease rarely proceeds acutely and superacutely. A conclusive diagnosis may be made on the basis of bacterioscopic, microbiological, and biological analysis of pathological material.

Emphysematous carbuncles in cattle somewhat recall the carbuncular form of anthrax; however, the nature of edematous development differs.

With emphysematous carbuncles, percussion produces a crepitant sensation coupled with a tympanic sound, while in the presence of anthrax emphysematous areas are non-crepitant; at first they are hard, and later they become doughy. In cases where it is difficult to make a diagnosis while the animal is still alive, pathological material is subjected to bacterioscopic, microbiological, and biological analysis, since these pathogens have differing cultural and biological properties.

Piroplasmosis is typified by seasonality of occurrence, by mandatory presence of carrier ticks, and by a positive response to specific chemotherapeutic agents and detection of *Babesia* in blood inspected under a microscope.

Laboratory Diagnosis

Sampling and Transporting Pathological Material

The carcasses of fallen animals must not be dissected when anthrax is suspected. If a suspicion of anthrax arises upon autopsy of such an animal--that is, when uncoagulated blood and typical infiltrates are detected and pigs exhibit inflammatory edema in the submaxillary region, autopsy is halted.

Blood is sampled from an incision made in the ear, and it is applied as a thick layer to a microscope slide previously cleaned with an ether-alcohol mixture. The smears are air-dried with the necessary precautions, after which they are wrapped in paper (parchment preferably), placed in a glass bottle with a ground-glass stopper or a metal can with a tight-fitting lid, and packed in a small wooden or metal box.

Blood for the smears may also be obtained from the jugular vein with a sterile syringe, which must be boiled together with the needle for 2 hours after use. The point of incision on the ear and the point at which blood is to be obtained from the vein are disinfected, and after the material is obtained, these points are seared with a red-hot spatula.

In addition to blood smears, an ear is taken from the fallen animal for laboratory analysis, from the side on which the carcass was lying. The ear is tied securely in two places and cut away. The cut is made between the ligatures, carefully so that blood would not spill on the ground. The ear is left tied. It is wrapped in parchment wetted with 3 percent carbolic acid solution or mercuric chloride solution (1:1000), and it is carefully packed in a wooden or metal box, which is sealed and sent to the laboratory by express messenger.

In the event of forced slaughter of an animal or if anthrax is suspected during autopsy performed for some other reason, autopsy is halted and a piece of the spleen or liver is carefully removed. From the carcasses of pigs, it is best to sample pieces of altered tissue in the vicinity of the pharynx or the retropharyngeal gland. When animals are subjected to forced slaughter, bacteremia tends to be subdued, and therefore more material should be taken for analysis.

The laboratory is furnished with material from the carcass of an animal that had just died, since material from an old carcass may be decayed, which usually makes it difficult and sometimes even impossible to isolate anthrax pathogen from it; anthrax microbes in a decomposed carcass undergo lysis, or they die due to the influence of putrefactive antagonistic microflora. Such material may be analyzed only by the precipitation reaction.

Microscopic Analysis of Pathological Material

Four or five drops of sterile physiological solution are applied with a Pasteur pipette to a slide bearing a thick layer of blood; the contents of the slide are mixed together, and two or three thin smears are made from the resulting mixture. Then the material is seeded in beef-extract broth and beef-extract agar in aseptic conditions, and the remaining smear is used to infect laboratory animals. To obtain material from the ear, the ligature is released, the ear is cut with sterile scissors, and a smear is made with the blood. Blood obtained from the ear and

diluted with physiological solution is seeded on nutrient media and injected into laboratory animals. Slides are applied under aseptic conditions to the cut surface of a spleen or liver sample to make a smear-print; nutrient media are also seeded under sterile conditions with material from these pieces. Then the pieces of spleen or liver are ground in a porcelain mortar with physiological solution at a ratio of 1 gm per 10 ml, and the resulting suspension is injected subcutaneously into white mice at a dose of 0.2 ml, into guinea pigs at a dose of 0.3 ml, and into rabbits at a dose of 0.5 ml. The remaining samples of pathological material are decontaminated in an autoclave at 1.5 atmospheres for 2 hours.

The prepared smears are fixed and stained with Gram stain or other water-soluble basic dyes. When viewed under a microscope in a Gram-stained smear, anthrax pathogen has the appearance of individual and paired rods, or short chains consisting of positively stained bacilli (Figure 14). As we know, anthrax bacilli form capsules in the animal body. Therefore detection of bacilli with clipped ends surrounded by a capsule is an extremely important sign in the diagnosis of anthrax. The microbial capsules are readily apparent under a microscope when the smears are treated with Giemsa, Rebiger, Ol't, Mikhin, Burtsev, and other stains (Figure 15).

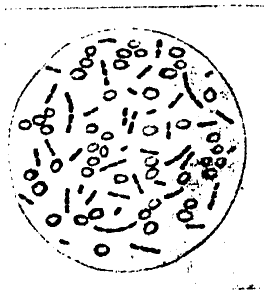

Figure 14. Anthrax Pathogen (Blood Smear)

Figure 15. Encapsulated Anthrax Agent Microbes

Anthrax diagnosis by bacterioscopic and biological methods may be extremely difficult inasmuch as the substance making up anthrax microbe capsules sometimes stains weakly and rejects the stain readily when the stain smear is washed with distilled or tap water. When smears of material from old carcasses are stained, the microbes may appear thinner, their ends are rounded, the morphological structure of the bacilli is disturbed, the bacilli appear pitted, and sometimes all that is left of the capsules is small fragments which naturally stain extremely poorly. This is especially evident in the hot part of the year, when it is difficult to get a good smear just one day after the animal's death. Therefore the sooner pathological material is obtained after the death of an animal for the smears and for microbiological and biological analysis, the more accurate the anthrax diagnosis would be.

Seeding Nutrient Media

Inoculation of BEA [beef-extract agar] in test tubes and Petri dishes and BEB [beef-extract broth] in test tubes with blood or parenchymatous organs from fallen laboratory animals should produce typical growth of anthrax microbes when cultured in a thermostat at 36-37° for 1 day. Dull gray, relatively large R-form colonies with a dark center and fringed periphery should arise on BEA in test tubes and dishes (Figure 16). At low magnification, colonies have the appearance of curls consisting of braids of long strands of microbes, which have come to be called "Medusa's heads" or "lion's manes". A flaky precipitate of microbial cells forms at the bottom of test tubes containing BEB, and the broth remains transparent. Pathogen in stained smears from cultures grown for 1 day on BEA and in BEB has the form of long strands consisting of rods with slightly rounded ends.

Figure 16. Colony of R-Form Anthrax Microbes

Anthrax pathogen grows in gelatin along the path of an injection needle in the form of an upside-down fir tree, with the medium liquefied in a funnel shape from the top downward (Figure 17). Milk coagulates in 2-4 days, followed by peptonization. Hemolysis does not occur in blood agar (in up to 15 percent defibrinated blood) (Table 2).

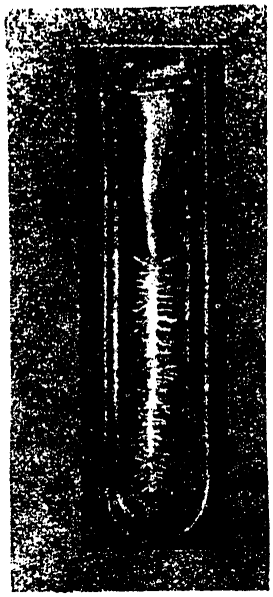

Figure 17. Growth of Anthrax Microbes Along Path of Injection Needle in Gelatin

Figure 18. RO-Form Anthrax Microbe Colonies

Table 2. Differential Cultural-Morphological and Biological Signs of *Bac. anthracis*

Bacillus	Relationship to Oxygen	Mobility	Spore Formation	Capsule Formation	Gram Staining	Liquid Media			
						Beef Broth (in Test Tube)	Broth With Blood	Milk	
								Coagulation	Peptonization
Bac. anthracis	+	-	+	+	+	Apparent growth after 6-7 hours. Broth remains transparent after 18-24 hours, with a flaky precipitate at the bottom of the test tube	No hemolysis	+	+
Bac. anthracoides	+	+	+	-	+	Broth turns cloudy, precipitate forms on the bottom	Hemolysis	+	+
Bac. pseudoanthracis	+	+	+	-	+	Broth becomes cloudy, crumbly precipitate forms on bottom, ring forms around test tube wall	Hemolysis	+	+
Bac. cereus (waxy)	+	+		-		Broth turns cloudy, crumbly precipitate. Film present; ring forms around wall of test tube	Hemolysis	+	+
Bac. mesentericus (potato)	+	+	+	-	+	Broth turns cloudy		+	+
Bac. megaterium (cabbage)	+	+	+	-	+	Broth turns cloudy, no film present. Insignificant precipitate		+	+

and Some Other Soil Bacilli

Solid Media		Pathogenicity		
Beef-Extract Agar (in Test Tube, Dish)	Gelatin (in Test Tube)	White Mice	Guinea Pigs	Rabbits
Light gray fibrous colonies recalling "Medusa's head" or a "lion's mane"	Slow growth along needle path in the form of an upside-down fir tree, with liquefaction of the medium occurring from top down on the 3d-5th day	Die after 1-2 days	Die after 2-3 days	Die after 2-4 days
Dull fibrous, curly, branched colonies	Stubbly growth thicker above; crater	Die in response to intraperitoneal infection at large doses	Nonpathogenic	Nonpathogenic
Round, whitish, curly, branched colonies		"	"	"
Round, whitish colonies, sometimes grayish yellow. Winding strands along the margins	Fast liquefaction. Horizontal outgrowth along needle path.	"	"	"
Wrinkled, slimy, white spotted colonies	Liquefaction coupled with formation of crater at top	Nonpathogenic	"	"
Dull-white, spotty, branched colonies	Funnel-shaped liquefaction from top down	"	"	"

Table 2 (continued)

Bacillus	Relationship to Oxygen	Mobility	Spore Formation	Capsule Formation	Gram Staining	Liquid Media			
						Beef Broth (in Test Tube)	Broth With Blood	Milk	
								Coagulation	Peptonization
Bac. subtillis (hay)	+	+	+	-	+	First turns cloudy; becomes transparent after film formation.	- +	+	+
Bac. mycoides (rhizomous)	+	+	+	-	+	Transparent broth, durable precipitate on bottom	+	-	+

Certain deviations may sometimes be encountered in the nature of growth of microbial cultures of anthrax material subjected to microbiological analysis. Individual rods, some making up short strands, may be seen in test tubes containing beef-extract broth, above the precipitate in the upper part of the medium, indicating diffuse growth of the anthrax culture. Sometimes RO-form colonies with smoother margins may appear in beef-extract agar (Figure 18). Pathogen from such colonies may be less virulent.

Spore formation is considered in analysis of pathological material grown on BEA. Although it is commonly accepted that spore formation terminates by the 3d day, strains in which it goes on for up to 4-5 days are encountered in practice. Spore formation may be easily established beneath a microscope with a crushed or hanging droplet or a smear treated with Peshkov's, Moeller's, Klein's, and other stains, obtained from a pure anthrax culture grown on beef-extract agar.

The Biological Diagnosis Method

Infection of laboratory animals with pathological material is the most accurate method of analysis. This method becomes necessary when the results of microscopic and bacteriological analysis are unclear. In general, biological tests with laboratory animals make up the concluding phase of anthrax diagnosis, mandatory for a conclusive diagnosis.

Laboratory animals are infected with a 10-15 percent suspension of pathological material in physiological solution or distilled water, or with pathological material grown in broth culture (undiluted), or with a physiological solution washing (5 ml) from agar culture (from an agar area of 5 cm^2). Animals are infected with the following doses: 0.1-0.2 ml for white mice, 0.3 ml for guinea pigs, and 0.5 ml for rabbits. Three to five white mice, two or three guinea pigs, and two rabbits are infected. White mice contracting anthrax die in 1-2 days, guinea pigs die in 2-3

Solid Media		Pathogenicity		
Beef-Extract Agar (in Test Tube, Dish)	Gelatin (in Test Tube)	White Mice	Guinea Pigs	Rabbits
Grayish white colonies with film forming on condensate	Film on surface of liquefied gelatin	Nonpatho-genic	Nonpatho-genic	Nonpatho-genic
Grayish white, loose colonies creeping through medium	Fast liquefaction coupled with crater formation	"	"	"

days, and rabbits die in 2-4 days. Animals are kept under observation for 10 days. A specific pathoanatomical pattern is observed upon dissection of the carcasses of killed animals--an enlarged spleen, in most cases typical gelatinous hemorrhagic edema of subcutaneous tissue of varying degree at the place of injection of the pathological material or culture, hyperemia of internal organs, and uncoagulated blood.

Encapsulated anthrax microbes are observed bacterioscopically in blood from the heart and parenchymatous organs. Seedings from the heart, spleen, and other organs produce typical growth of these microbes.

In laboratory practice, only white mice and, in the best case, guinea pigs are often infected by pathological material or by cultures grown from it. However, it is known that anthrax agent strains varying in virulence occur in nature, and that their high virulence can be determined only by infecting rabbits with pathological material or cultures produced from it; in this case a positive diagnosis of anthrax should be confirmed only on the basis of the death of such rabbits. Relatively large doses would be enough to determine the virulence of isolated pathogen cultures with rabbits. However, in order to determine the degree of virulence of a culture, minimum and maximum doses must be used.

In their book "Anthrax", P. N. Burgasov, B. L. Cherkasskiy, L. M. Marchuk, and Yu. F. Shcherbak (1970) point out that the virulence of anthrax microbes may be revealed with a dose of 10,000 or 100,000 spores per rabbit weighing 2-2.5 kg, while three different doses--100, 1,000, and 10,000 spores per rabbit at a volume of 1 ml--must be injected toward the end of the experiment in order to establish the degree of their virulence. Each dose is injected into two rabbits. The spore culture is grown 3-4 days on beef-extract agar, Hottinger's agar, or agar medium made from over-cooked casein. Spore culture washed from agar medium is diluted in 0.15 M NaCl solution to standard GKI No 10 turbidity, which corresponds to about 100 million anthrax spores. The necessary doses for infection of rabbits are obtained from the prepared dilution.

Experimental rabbits are kept under observation for 10 days.

A study of the behavior of anthrax culture strains in rabbits showed that their virulence is far from identical. Some strains may kill rabbits when introduced at a minimum dose, for example 100 or 1,000 spores. Other strains may cause rabbits to die only at doses of 5,000-10,000 spores. This indicates a need for establishing optimum doses, such that the virulence of a culture produced from pathological material obtained from the carcass of a fallen animal or from some other material, for example soil, water, products of animal origin, and so on, could be determined. In such cases strains causing the death of rabbits at doses of 100 and 1,000 spores are said to be highly virulent, while strains causing the death of rabbits at doses of 5,000-10,000 spores are moderately virulent. Of course this gradient in the virulence of anthrax microbes is relative, but it does permit practical veterinarians to assess how virulent an anthrax culture causing the death of an animal is.

Anthrax microbes are cultivated until complete spore formation (90-100 percent spores) on beef-extract agar or on Hottinger's agar for 4 days. The completeness of spore formation is determined by microscope with crushed or hanging droplets or with Aujeszky-stained smears. The spore culture is washed with physiological solution until a suspension is acquired with a concentration of about 100 million spores--optical turbidity standard GKI No 10. Following the appropriate dilutions with physiological solution, the required doses are obtained from this suspension. Rabbits are infected with the spore cultures at doses of 100, 1,000, and 10,000 spores in a 1 ml volume, subcutaneously in the abdominal area. Two rabbits weighing 2-2.5 kg each are used with each dose. The rabbits die of anthrax in 2-4 days, while rare individuals may die 5-7 days after infection. Autopsy of dead rabbits reveals a specific pathoanatomical pattern. Special staining reveals encapsulated microbes in smears prepared from the blood and parenchymatous organs of fallen animals. A pure, typical culture of anthrax pathogen is isolated from seedings of blood from the heart and from parenchymatous organs.

The Serological Diagnosis Method

One of the principal ways for diagnosing anthrax is to analyze material by the precipitation reaction. This reaction is especially convenient and dependable when the delivered material is old and decayed, since it is impossible to isolate anthrax pathogen from such material by the bacteriological method due to lysis of the microbes. The precipitation reaction is used with fresh pathological material from deceased animals, and to test microbial cultures isolated from delivered material. This reaction is used especially extensively to test raw hides from the public at large and all imported hides for anthrax.

The following materials are used in the precipitation reaction: precipitating anthrax serum, antigen (precipitinogen) from the material under analysis, and standard bacterial anthrax antigen for the control.

Precipitating anthrax serum was first obtained by Ascoli and Valenti (1910) via intravenous hyperimmunization of asses by a weakly virulent culture of anthrax microbes. This serum came into use in the precipitation reaction to diagnose anthrax, and this method has come to be called the Ascoli precipitation reaction.

In Russia, S. A. Alekseyev obtained precipitating anthrax serum in 1912 by intravenous hyperimmunization of horses with Lange's vaccine No 1.

The serum acquisition method was subsequently improved. S. K. Bezzubets (1920-1927) used a more-intensive method to hyperimmunize horses, utilizing a culture of the vegetative form of Tsenkovskiy's vaccine No 1 as the antigen. Precipitating anthrax serum obtained in this manner was insufficiently specific, in connection with which further efforts were made to improve it.

In 1940 N. M. Nikiforova and M. I. Anan'yev developed a new method for obtaining precipitating anthrax serum by means of intravenous hyperimmunization of horses with virulent anthrax vegetative culture killed with formalin. This serum possessed high specificity, but it was not active enough, and consequently it had a short shelf life--1 year, which made it necessary to develop an improved method for acquiring precipitating anthrax serum.

In 1951-1952 S. G. Kolesov and V. N. Grachev developed a new method for obtaining precipitating anthrax serum. The use of weakly virulent and immunogenic strains of anthrax microbes is at its basis. Live cultures of the vegetative form of GNKI anthrax vaccine strain Sh-15, strain No 916-1, Tsenkovskiy vaccine strain No 2, and strain No 94 were used for hyperimmunization of horses. The first two strains are capsuleless, and the other two have weak capsulogenic properties. Horses previously subjected to five-time subcutaneous immunization with anthrax vaccines with the goal of creating background immunity were hyperimmunized by injecting culture intravenously. Antigen was prepared separately for this purpose from the four strains indicated above by growing them in pea agar for 20-22 hours in a thermostat at 36-37°. Antigen with a concentration of 2-3 billion was prepared from the culture, washed with physiological solution; this antigen was injected into the horses in increasing doses. The injections of strains were alternated--that is, one strain was introduced with each injection.

Bacterial anthrax antigen is prepared from a virulent anthrax agent strain by growing it for a day in BEA. The obtained culture is autoclaved for 30 minutes at 120° and dried to constant weight. The dried bacterial mass is diluted with physiological solution at a ratio of 1:3000-1:5000, and filtered through sterilizing filter plates, after which it is packed into vials and autoclaved once again at 120° for 30 minutes.

After each series of precipitating anthrax serum and standard bacterial anthrax antigen is prepared, it is tested for sterility, activity, and specificity, in accordance with the instructions on making and testing these biological preparations. Serum must produce a positive precipitation reaction with standard bacterial anthrax antigen in 20 to 40-50 seconds, while serum reacting with hide anthrax antigen used to test serum at a biological plant should produce a positive reaction in 2-3 minutes. The reaction with antigens obtained from the hides of slaughtered healthy animals should be negative in 15 minutes. The precipitation reaction should be negative in 15 minutes also with antigens from anthracoid and pseudoanthrax bacilli, prepared by the same method used to acquire bacterial anthrax antigen, except for the use of a maximum dilution of 1:10,000.

The precipitation reaction is used to test pathological material for anthrax. For this purpose the delivered material--pieces of skin (if the carcass had not been

dissected) or muscle--is first decontaminated by autoclaving at one atmosphere for 60 minutes; then it is cut into 3 × 3 mm pieces, covered with physiological solution at a ratio of 1 gm material per 10 ml solution, and extracted at 16-18° for 16-18 hours. The extract is filtered until transparent through asbestos cotton having a neutral pH.

According to L. Ye. Naymushina and other researchers the order of analysis of raw hides and furs for anthrax differs little in principle from analyzing pathological material. It consists of the following stages: sterilizing, grinding, and extracting the samples, filtering the extracts, combining the components, and determining the analysis results--that is, reading the precipitation reactions.

Raw hides and furs preserved in different ways (freshwater-dried, frozen, dry-salted, wet-salted) are sterilized separately in an autoclave. The samples are sterilized at 1.5 atmospheres for 30 minutes, or at 1 atmosphere for 1 hour.

Following sterilization, the samples are meticulously pulverized (down to 3 × 3 mm particles) by automatic or hand-operated instruments, and wool is first removed from hides with scissors. The smaller the sample particles are, the better and more fully is the antigen extracted.

Samples of freshwater-dried, dry-salted, frozen, and steamed hides are pulverized and placed in individual cans as 1 gm batches, and covered with 10 ml physiological solution containing 0.3 percent crystalline carbolic acid. Extraction is performed at 15-16° for 16-20 hours. The obtained sample extracts are filtered through asbestos wool packed in a funnel with a diameter of 39 mm firmly enough to produce a completely transparent fluid after 1-2 hours of filtration. If the resulting filtrate is not transparent enough, it is refiltered.

Strict records are kept on the material samples during their preparation: The samples are numbered, and the appropriate notes are made in logs.

The precipitation reaction is performed with completely transparent components. Precipitating anthrax serum is allowed to settle for 10-14 days, or it is filtered through asbestos wool until transparent. When dry-salted and wet-salted raw hides are analyzed, 3-4 percent chemically pure sodium chloride is added to the precipitating serum in order to increase its specific weight.

The filtered sample extracts are layered over precipitating serum previously poured into test tubes; this procedure is performed very carefully to keep the components from mixing together, since otherwise the precipitation reaction may not occur. If a sharp boundary does not develop at the interface of the components, the antigen extract must be layered over once again.

The precipitation reaction results are read from samples of steamed, freshwater-dried, and frozen hides after 10-12 minutes, and not later than 15 minutes from the moment the components are combined; the reading time for dry-salted hides is 30 minutes, and that for wet-salted hides is 1 hour. The precipitation reaction is said to be positive (+) if a grayish white disc (ring) appears at the indicated time at the interface of the extract and serum. No traces of precipitation are evident at the interface of the component in a negative reaction (-). If there is a weakly pronounced ring, clouding, and so on, the reaction is said to be doubtful.

Detection of Anthrax Microbes in the Environment

Anthrax pathogen may be detected in soil, in places where sick animals had been maintained prior to their death, in places of the death, slaughter, and burial of the carcasses of anthrax-stricken animals, in the water and sediments of open-air water basins receiving waste water contaminated by sick and dead animals, on objects used to care for sick animals, in the dust and air of buildings in which such animals had been maintained, on feed, in raw materials of animal origin, and in semifinished products and food products obtained from animals stricken with anthrax. This is why soil, water, sediment, forage, meat, hides, wool, hair, bristle material, protective clothing, harnesses, and other such materials must be tested for anthrax. In our method, soil samples are ground and mixed, small batches (3-5 gm) are placed in 2 or 3 flasks, covered with sterile distilled water or physiological solution at a 1:10 ratio, carefully mixed, and heated for 20 minutes at 75-80°. Then they are centrifuged for 10-15 minutes at 2,500-6,000 rpm. The supernatant is suctioned off, and the precipitate is seeded on BEA in test tubes and Petri dishes, carefully irrigating the surface of the agar with the material under analysis, and in test tubes containing BEB. The cultures are grown for 24 hours in a thermostat at 36-37°. Laboratory animals are infected with the remaining material, which is injected subcutaneously at doses of 0.5 ml for white mice, 1 ml for guinea pigs, and 2 ml for rabbits.

Water samples of not less than 50 ml in each flask are heated by the method described above and centrifuged for 10-15 minutes; the resulting precipitate is inoculated into nutrient media, after which the cultures are grown in a thermostat. Laboratory animals are infected with the precipitate.

Forage samples weighing 25-30 gm each are cut into small pieces with scissors, placed in flasks, covered with sterile distilled water or physiological solution (100 ml), thoroughly shaken, heated at 75-80° for 20 minutes, poured through a funnel with a gauze filter into centrifuge tubes, and centrifuged for 10-15 minutes. The precipitate is inoculated into nutrient media and injected into laboratory animals.

Samples of wool, bristle, and hair weighing 5-7 gm each are separately cut up into fine pieces with scissors in a mortar, covered with sterile distilled water or physiological solution (25-30 ml), and thoroughly ground with a pestle, after which the contents are suctioned off, heated at 75-80° for 20 minutes, poured into test tubes, and centrifuged. The resulting precipitate is inoculated into nutrient media to permit growth of anthrax microbes, and injected into laboratory animals.

Pure anthrax microbe cultures can be seen in the nutrient media: R-form colonies on BEA, and a typical flaky precipitate at the bottom of test tubes containing BEB, with the upper part of the broth remaining transparent. Strains are encountered exhibiting diffuse growth in broth; such cultures consist of individual and paired bacilli, or short chains.

Sometimes a pure anthrax microbe culture may not be obtained in nutrient media. In such cases BEA cultures are carefully examined with a magnifier or a microscope at low magnification in order to find the pure anthrax culture. If this effort is unsuccessful, the mixed culture is subdivided and reinoculated at different dilutions on agar in Petri dishes, and then anthrax pathogen is isolated from individual colonies exhibiting characteristic form.

The carcasses of the fallen laboratory animals infected with the material under analysis are autopsied. BEA and BEB cultures and smears are made from blood taken from the heart and parenchymatous organs. Typical changes are observed in parenchymatous organs from laboratory animals killed by anthrax, and the cultures produce pure anthrax microbe colonies.

In the method developed by the All-Union Scientific Research Institute of Veterinary Medicine, soil to be analyzed is cleaned of roots and pebbles, mixed, divided into 10 gm samples, placed into flasks, and covered with 50 ml distilled water. The flasks are plugged with rubber stoppers and agitated for 25 minutes, after which they are allowed to stand for 5-8 minutes to allow large particles to settle. The supernatant is seeded at a dose of 0.2 ml on BEA in three or four bacteriological dishes. The samples are left covered for 10-15 minutes to dry out, after which the material under analysis in each dish is inoculated in separate fractions into two or three new dishes containing BEA. The inoculated dishes are placed in a thermostat at 37° for 24 hours. After this, typical anthrax colonies and suspected colonies are separated out. The supernatant is injected into white mice. The mice are given a 0.5 ml subcutaneous dose in the inguinal region and observed for 10 days. Mice that die are autopsied, and samples from their organs are cultured in nutrient media. When the results are positive, typical growth of anthrax cultures is observed.

P. N. Burgasov, B. L. Cherkasskiy, L. M. Marchuk, and Yu. F. Shcherbak recommend the following methods for detecting anthrax pathogen in the environment. Soil is divided into 30 gm samples that are covered with 100 ml water and shaken for 5-10 minutes. The resulting suspension is allowed to settle for 1-2 hours. The supernatant is decanted and mixed with a quantity of sterile physiological solution sufficient to make up 1 liter.

One-liter washings are made from environmental objects to be analyzed, while extracts, also 1 liter in volume, are made from hides, wool, hair, and so on.

If the liquid under analysis exhibits pronounced opalescence or if it contains suspended particles, it must be filtered through sterile gauze, cotton, or coarse filter paper, which could also be analyzed subsequently. Water or supernatant to be analyzed is divided into 500 ml samples. One batch of samples is heated for 30 minutes at 65-70°. The unheated liquid is passed separately in equal portions through four No 3 membrane filters in order to concentrate the microorganisms. When the quantity of liquid is insufficient, 10 ml portions may be passed through each filter. The deposits of two filters are applied to the surface of 0.7 percent beef-extract agar in Petri dishes and heated in a thermostat at 37° for 3 hours. The resulting culture is used in luminescent serological analysis. Washings are made from the two other membrane filters into sterile Petri dishes by applying 1 ml 0.15 M NaCl solution to their surface with a pipette. Then the washings are inoculated on GKI medium in order to reveal capsule formation by the microbes, and injected into white mice. The second part of the heated water or supernatant is also filtered through membrane filters and analyzed as indicated above.

Anthrax microbes are differentiated from soil spore-forming aerobic Gram-positive microorganisms in samples of soil, water, forage, and other materials by, in addition to the methods listed above, the cultural, morphological, and biological properties described in Table 2.

Quick Diagnosis Methods

These methods are based on the anthrax pathogen's capability for synthesizing capsules in nutrient media containing a significant quantity of blood serum protein.

In 1960-1962 V. R. Arkhipova proposed a quick method for revealing anthrax microbe capsules on GKI medium. The medium is prepared as follows: 40 ml bovine serum inactivated at 56° for 30 minutes are added to 60 ml sterile Hanks' solution in a flask. The ingredients are carefully mixed and brought to pH 7.2 with sodium bicarbonate. The obtained medium is poured under sterile conditions in 2 ml quantities into test tubes, which are then plugged with rubber stoppers. The medium may be stored in this form at 4° for a long period of time without deterioration of its qualities.

Any pathological material can be seeded on GKI medium (with a pipette or a loop), and it may be used as a medium for test suspensions obtained in analysis of soil, water, and other materials (feed, wool, hair). Inoculated test tubes are placed in a thermostat at 37°. It is presumed that most anthrax microbes form capsules in this medium within 16-18 hours. Some microbes form a capsule as early as 1-3 hours after the cultures are placed in the thermostat. Smears are prepared from the resulting cultures. The smears are dried and fixed with methyl alcohol for 5 minutes, after which they are stained through filter paper with Loeffler's blue for 3-5 minutes and observed under a microscope, first at low magnification and then with an immersion lens. Anthrax microbes stain bright blue, while capsules turn pale pink.

If Hanks' solution is unavailable, according to R. A. Saltykov capsules could be revealed by inoculating the material under analysis into Hottinger's broth to which 40 percent inactivated serum is added.

B. Ya. Mikhaylov, G. I. Rozhkov, and A. L. Tamarin (1960) proposed a method for detecting capsules using coagulated serum. It is based on the capability of anthrax microbes for forming capsules in this medium in the presence of carbon dioxide (CO_2).

Water can be tested for presence of anthrax pathogen by filtering it through membrane filters No 2 and No 3 (a method suggested by Ya. Ye. Kolyakov and A. D. Melikhov). The filters are first sterilized by boiling for 30 minutes, after which water samples are pumped through the filters at a rate of 1 liter for every 7-10 membrane plates. The latter are placed together with the adsorbed microbes into sterile broth containing 10 percent normal equine serum and then held in a thermostat for 5-6 hours for growth. The resulting culture is boiled for 30 minutes and then filtered through asbestos wool; the filtrate is tested with the precipitation reaction using precipitating anthrax serum. The culture samples are simultaneously inspected microscopically, and if necessary they are tested biologically.

E. N. Shlyakhov and Ye. V. Gruz (1960) proposed a method for revealing anthrax microbe capsules *in vivo*. According to this method washings from a membrane filter bearing material being analyzed for anthrax are injected intraperitoneally into 10 white mice at a dose of 0.1-0.2 ml. Two mice are killed 30, 60, 120, and 180 minutes after injection, and the remaining two are kept until the end of the experiment as controls. If the material being analyzed contained anthrax microbes, the

mice would die in the usual time. Killed mice are autopsied, and smears and prints are made from peritoneal exudate and organs (spleen, liver, kidneys). The smears and prints are fixed and stained to reveal capsules. A diagnosis of anthrax is made if encapsulated forms of the pathogen are detected in the smears.

Differential Diagnosis

Anthrax pathogen *Bac. anthracis* has some cultural and morphological properties similar to those of other representatives of genus *Bacillus* (*Bac. anthracoides*, *Bac. cereus*, *Bac. pseudoanthracis*, and so on). The "necklace" reaction, the bacteriophage test, and luminescent microscopy are performed to differentiate anthrax pathogen from these microorganisms.

The "necklace" reaction was first described by Jensen and Kleemeyer in 1953.

Later, its use was reported by Ya. Ye. Kolyakov (1960), E. N. Shlyakhov (1967), S. G. Kolesov and V. V. Arkhipov (1968), and others. The "necklace" reaction is based on the effect penicillin has upon anthrax pathogen cells in the initial phase of their development.

To reveal the reaction, a broth culture of the microbe under analysis is seeded on BEA in Petri dishes after 3 hours of growth. Prior to this, penicillin is added to the BEA at a dose of 0.5 to 0.05 units per milliliter of nutrient medium, which is then placed in a thermostat for 3 hours at 37° for growth. Anthrax microbes growing in BEA containing penicillin take on a spherical shape, and their chains recall a "diamond" necklace (Figure 19). Spore-forming saprophytic aerobic microorganisms grow in their usual forms in similar conditions.

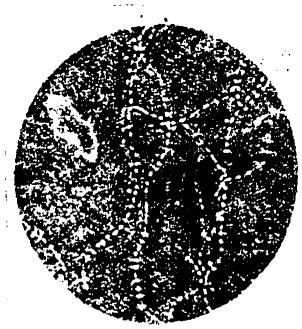

Figure 19. The "Necklace" Reaction

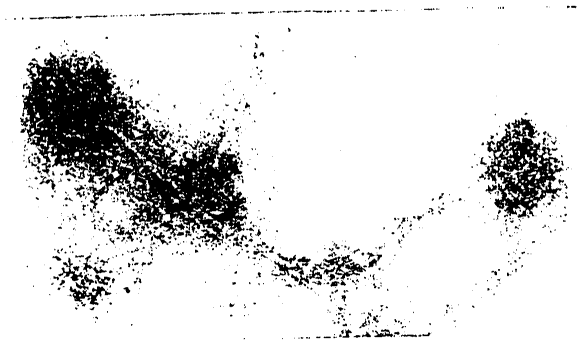

Figure 20. Anthrax Bacteriophage (From Ye. N. Levina)

The bacteriophage test: Two anthrax bacteriophages are produced by our country's biological industry: The VIEV's [All-Union Institute of Experimental Veterinary Science] "K" bacteriophage (Figure 20) (A. Ya. Meshcheryakov, 1961), and the MVA's [Moscow Veterinary Academy] "Gamma" bacteriophage (Ya. Ye. Kolyakov and V. A. Bayrak, 1962).

The anthrax bacteriophage test is based on interaction of the bacteriophage with anthrax culture resulting in lysis of the latter. This reaction is highly specific, and it is used to identify the anthrax pathogen and differentiate it from pseudo-anthrax bacilli.

VIEV "K" anthrax bacteriophage is made with avirulent strain No 643/11 or "Davis" atypical strain, each of which is highly sensitive to the bacteriophage. A broth culture of one of these strains is inoculated into a flask containing yeast broth with a pH of 7.2-7.4, and incubated for 18-20 hours at 37°. Then this culture is seeded in (matrovyye) flasks containing yeast agar and placed in a thermostat at 37° for 5-6 hours. After this time the culture is washed off with physiological solution and brought to a concentration of 1-5 billion microbial cells per milliliter, as determined by the optical standard. Next the bacteriophage is prepared by one of two methods. In the first method agar culture washed with physiological solution is diluted with yeast broth to a concentration of 1 billion microbial cells per milliliter according to the optical standard, and then the bacteriophage is introduced into these flasks at a rate of 1:2-1:10 in relation to the volume of the microbial cell suspension. After this, the flasks are shaken and placed in a thermostat in tilted position with the agar slanting downward, at a temperature of 37° for 10-18 hours. In the second method bottles containing yeast broth are seeded with a suspension of 5-6 hour agar culture and 10-100 ml bacteriophage at a rate of 1 liter of medium per 100-200 ml suspension. Then these bottles are placed in a thermostat for 10-18 hours at 37°. Bacteriophage obtained by one of these methods is filtered through SF asbestos plates. Each phage series is tested for purity, activity, and specificity. The Appelman titer of the bacteriophage is not less than 10^{-8}-10^{-9}.

The following methods are recommended for identification of anthrax pathogen: the micromethod, the test tube method, the luminescent-serological method, and the phage titer growth reaction method. The first two methods are the most acceptable to practical workers.

Analysis by the micromethod involves the use of bacteriological dishes filled with an even layer of 1.5-2 percent BEA 3-5 mm thick. The dried surface of the agar is divided into several sections. Each sample of prepared material--a suspension of 3-6 hour culture having a concentration of 125-500 million microbial cells--is applied with a bacteriological loop to three sections of one vertical row, and dried for 5 minutes. Then one drop of undiluted bacteriophage is applied with a Pasteur pipette to the first two sections. The dishes are rocked to distribute it over the entire surface of each section. The third section is the control for the culture under analysis. The dishes are held for 10-15 minutes at room temperature to permit drying, after which they are placed in a thermostat at 37° with their covers downward. The results are read after 4-6 hours or 12-24 hours. In the first case the sections are viewed with a microscope at low magnification, and in the second they are examined with the unaided eye or a magnifier. The results are determined from the presence or absence of cell lysis in sections containing bacteriophage, in comparison with culture growth in the control section. If lysis is noted in sections containing bacteriophage, the culture contains anthrax pathogen.

The test tube method involves the use of yeast broth poured into four test tubes in 4.5 ml batches. A 0.5 ml volume of bacteriophage having a titer of 10^{-8}-10^{-9} is added to the first and fourth tubes. After shaking, 0.5 ml are transferred from the first to the second test tube. Then 0.1 ml suspension of the culture under analysis are added to each of the first three tubes. The third test tube serves as the culture control, and the fourth is the bacteriophage control.

The results are read after 4-6 hours of incubation in a thermostat. If the culture contains anthrax pathogen, growth would be evident in the third test tube and absent in the first two, in which the medium would remain transparent. The fourth test tube should always be transparent.

Anthrax strain 1007 is used to prepare MVA "Gamma" phage. A day-old agar culture of this strain with a concentration of 1.5-2 billion microbial cells per milliliter, as determined by the optical standard, is inoculated into flasks containing BEB at a rate of 100 ml medium per milliliter culture and milliliter of source phage. The contents of the bottles are mixed, and then the bottles are placed in a thermostat for 24 hours at 37°. After this time the medium is filtered through sterilized SF filter plates. The resulting phage is tested for purity, activity, and specificity. Phage activity must be not less than 10^{-7}. The phage is used to determine anthrax pathogen, and to indicate its presence in fresh pathological material by the drip method on a tilted agar surface. For this purpose one droplet of the culture under analysis or of a suspension of the pathological material, initially ground in a mortar with physiological solution, is applied to the surface of agar in a test tube. The material is distributed uniformly over the entire surface of the agar, and then the test tubes are placed in a thermostat for 15 minutes at 37°. Next one drop of undiluted bacteriophage is added to each test tube, and the test tubes are placed in the thermostat for 6-18 hours.

If the result is positive, then after this time there would be no culture growth along the path of the draining droplet, while continuous culture growth would be observed in the control test tube, to which the phage had not been applied. If a "track" is absent, the culture is not anthrax.

Diagnosis with fluorescing antibodies: M. N. Meysel', Ye. A. Kabanova, et al. (1957) and Ye. N. Levina (1958) were among the first to test the fluorescing antibody method as a means for revealing anthrax pathogen. A significant number of studies were subsequently conducted demonstrating that the fluorescing antibody method is a sensitive test for revealing and identifying vegetative anthrax bacillus cells (P. I. Pritulin, N. A. Kuz'min, 1959; L. F. Dolgov, 1960; Ye. N. Levina, 1962; M. A. Shestochenko, 1967; N. A. Kuz'min, 1968, etc.).

However, luminescent anthrax serums turned out to be insufficiently specific, causing luminescence of spore-forming saphrophytic bacilli having group antigens in common with those of anthrax microbes. Therefore in order to raise the specificity of the immunofluorescence reaction with anthrax, a method was proposed in which related species of saphrophytic bacilli or dry anthracoid microbial powder, prepared by acetone-ether drying, are adsorbed onto serum (P. I. Pritulin and N. A. Kuz'min, 1959; D. Sh. Akhmerov, 1962; V. M. Podkopayev, 1964; N. A. Kuz'min, 1968, 1971, etc.).

Cherry and Freeman (1958) used luminescing anticapsule serum to reveal encapsulated forms of anthrax bacilli in the bodies of experimental animals. This method is sufficiently specific, and it is highly sensitive (Ye. N. Levina, R. B. Gol'din, et al., 1972).

The immunoflorescence method made it possible to reveal species-specific heat-stable antigens of spore forms of anthrax bacilli, differing from the antigens of anthrax pathogen's vegetative forms and the spore forms of saphrophytic aerobes. This was the starting point for using luminescent antispore serums to reveal and differentiate spore forms of anthrax microbe (Ye. N. Levina, 1967).

Dowle and Hansen (1961) attempted to avoid the difficulties associated with the common antigenic structure of anthrax bacilli and anthracoid bacilli by successively processing stained microbes with anthrax-specific gamma-phage and with fluorescing antiphage serum depleted by an anthrax bacillus culture. This method turned out to be promising. Presently the fluorescing antibody method using adsorbed serum is believed to be one of the dependable tests for early detection and identification of vegetative forms of anthrax microbe.

The immunofluorescence method may be used in the following two variants for laboratory diagnosis of anthrax:

The direct immunofluorescence reaction;

the luminescent-serological test using a phage-fluorescent antiphage system.

The first variant makes use of adsorbed anthrax luminescent serum, which may be obtained from a biological materials plant. This method reveals unencapsulated vegetative anthrax bacillus cells, and it may be used to identify isolated cultures; however, it can only be used for orientation purposes, since additional information would be required concerning virulence, capsule formation, susceptibility to lysis by specific phage, and so on.

Smears are prepared from 18-24 hour cultures, or impressions are made of pathological material. These prints and smears are dried in air and fixed for 15 minutes in 96 percent ethanol (they may be fixed for 5 minutes in methanol), after which they are rinsed with physiological solution and dried. Then the vial is opened, and dry luminescent serum is diluted by the appropriate volume of distilled water, after which the working dilution indicated on the vial label is prepared with physiological solution.

The preparations are placed in Petri dishes lined with moistened filter paper. Luminescent serum is applied, and the dishes are held at room temperature for 20 minutes. After this the preparations are given two thorough washings with physiological solution, they are rinsed with distilled water, air-dried, and observed under a luminescent microscope with a 90 × 1.25 immersion objective and a 5 × ocular.

Anthrax bacilli processed with homologous luminescent serum are typified by bright yellowish green luminescence along the cell periphery, and they exhibit typical morphology (Figure 21).

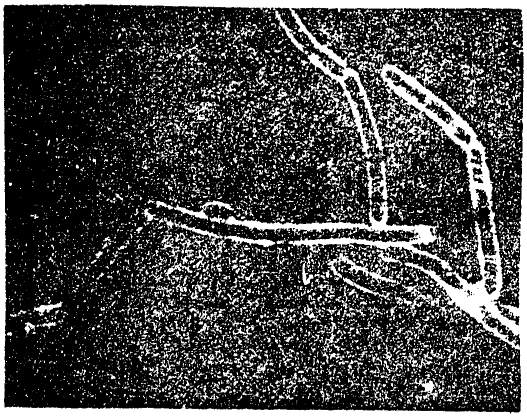

Figure 21. Anthrax Microbes Processed With Luminescent Anthrax Serum

The intensity of luminescence of microbial cells is graded in crosses: ++++ very bright luminescence around the periphery of the cell, clearly contrasting with the dark cell body; +++ bright luminescence of the cell periphery; ++ weak luminescence of the cell periphery, contrasting insignificantly with the cell body; +, ± very weak luminescence of the cell outline.

Typical morphology of the bacteria and luminescence graded from ++++ to ++ are sufficient for a tentative conclusion that anthrax pathogen has been detected.

The luminescent-serological test employing a phage-fluorescent antiphage system is based on the phenomenon of specific adsorption of anthrax phage particles upon sensitive homologous microbial cells, their specific reaction to antiphage fluorescent serum, and subsequent revelation of the pathogen by luminescent microscopy. Specific

luminescence of bacterial cells in preparations processed by the phage-luminescent antiphage system indicates presence of anthrax pathogen in the material under analysis.

This variant of the luminescent-serological method is indirect; it is highly sensitive and specific.

The diffuse precipitation reaction (DPR) was used for the first time by Thorne and Belton (1957) as a method for titrating protective antigen and antibodies homologous to it. Many domestic and foreign researchers subsequently studied the same method for the purposes of the DPR.

Concurrently, attempts were undertaken to use this reaction as a diagnostic serological test for anthrax, mainly as a means for indicating presence of the pathogen and for differentiating it from aerobic spore-forming saprophytes.

Mathois (1962) concluded that the precipitation reaction in agar gel is fully suited to anthrax diagnosis, and that it is more convenient than classical Ascoli thermal precipitation: Absolutely transparent ingredients are not required, the same controls may be employed, and the DPR is more specific and sensitive than the test tube reaction. Moreover the reaction can also be performed with edematous fluid and native pathological material obtained from fallen animals. Evidence of this can also be found in observations by V. V. Akimovich, N. S. Goncharova, and I. N. Smirnova (1962), and A. M. Adlan (1966).

It is much more difficult to use this reaction to differentiate anthrax bacillus in mixed cultures, since spore-forming aerobic saprophytes, primarily *Bac. cereus*, have common antigenic components (V. V. Akimovich, I. N. Zemtsova, et al., 1964). Nevertheless E. N. Shlyakhov, S. A. Shvarts, and T. A. Burdenko (1964) came to the conclusion that adsorbed precipitating anthrax serum may be used in the gel diffuse precipitation reaction to identify anthrax microbe.

N. M. Kalashnik (1967) made a detailed study of the possibility for detecting anthrax agent with the double immunodiffusion reaction in agar. The author concluded that the DPR may be used successfully to analyze raw hides, and especially samples producing a doubtful Ascoli precipitation reaction. This method reveals anthrax antigen in absolutely all raw hide samples. Similar results were obtained with chunks of spleen, liver, and edematous tissues from animals killed by anthrax.

The (Ukhterloni) method is the most acceptable. It is based on the principle of double diffusion in agar gel, Difco agar being the best: It does not require initial processing. A 1 percent transparent gel is prepared with physiological solution or phosphate buffer (pH 7.3).

Merthiolate is added at a 1:10,000 ratio to prevent bacterial growth.

Other types of agar must be subjected to special processing. First 30 parts distilled water are mixed with one part agar, 0.5 percent calcium chloride is added, and the mixture is boiled until the agar melts. The hot agar is filtered through two layers of gauze and then poured into vessels with a flat bottom as a layer 1 cm thick. After the agar cools, it is cut into square (1 × 1 cm) chunks and rinsed by a stream of tap water for 3 days in a glass jar covered with gauze. Then the agar

is held for another day in several changes of distilled water. The water is drained away, the agar is melted in a water bath, and equal quantities of 1.6 percent sodium chloride solution and 1:10,000 merthiolate are added to it.

Agar prepared in this fashion is poured into flasks and held at 4° until use. Just before the reaction the agar is melted in a water bath, poured into Petri dishes in 18 ml batches, and cooled until hard. Craters with a diameter of 8 mm are made in the hardened agar plate with a drill or with a special stamp. These craters are arranged in groups of 3, 5, and 7, or as parallel rows. The distance between rows should be 6-8 mm. In order that the ingredients of the reaction would not leak beneath the agar plate, one or two drops of melted agar are placed on the bottom of each crater.

The craters are filled with serum and antigen-containing material. When the craters are arranged in groups of five and seven, serum is placed in the central crater and antigens are dropped into the peripheral craters; when the craters are in parallel rows, one row is filled with serum, and the opposite row is filled with antigens.

Plant-produced precipitating anthrax serum serves as the antibody source, and the antigen-containing material may vary: broth or agar (live and killed) cultures, or chunks of skin, parenchymatous organs, and blood from animals killed by anthrax. Putrefactive decomposition of tissues does not have an influence on the precipitation reaction results.

Filled dishes are placed in a moist chamber (this step may be ignored) and left at room temperature or in a thermostat at 37°.

The reaction is sufficiently distinct after 24 hours, manifesting itself as formation of a well-delimited precipitation line. Lines of identical serological systems merge, while those of unidentical systems cross.

When the reaction is performed with protective antigen, the precipitation lines may appear in 3 days, but not later than 5.

The last two methods of anthrax diagnosis are not extensively employed as yet; however, since they are more specific, their use should increase as practical veterinarians assimilate them.

BIBLIOGRAPHY

1. Alekseyev, S. A., AVN, Book 8, 1912.

2. Arkhipova, V. R., "Trudy Vsesoyuznogo nauchno-issledovatel'skogo instituta veterinarnoy sanitarii" [Works of the All-Union Scientific Research Institute of Veterinary Medicine], Vol 21, 1962, p 172.

3. Bezzubets, S. K., "Trudy I-go Vsesoyuznogo veterinarnogo nauchno-organizatsi-onnogo s"yezda" [Works of the First All-Union Veterinary Scientific Organizational Congress], 1927, p 1.

4. Burgasov, P. N., Cherkasskiy, B. L., Marchuk, L. M., and Shcherbak, Yu. F., "Sibirskaya yazva" [Anthrax], Moscow, 1970.

5. Kolesov, S. G., and Grachev, V. N., "Trudy Gosudarstennogo nauchno-kontrol'nogo instituta" [Works of the State Scientific Control Institute], 1955, p 5.

6. Kolesov, S. G., and Arkhipov, V. V., "Diagnostika infektsionnykh i protozoynykh bolezney sel'skokhozyaystvennykh zhivotnykh" [Diagnosis of Infectious and Protozoan Diseases of Agricultural Animals], Moscow, 1968.

7. Kolyakov, Ya. Ye., and Bayrak, V. A., "Materialy 8-y nauchnoy konferentsii po infektsionnym i invazionnym zabolevannyam sel'skokhozyaystvennykh zhivotnykh" [Proceedings of the 8th Scientific Conference on Infectious and Invasive Diseases of Agricultural Animals], Moscow, 1962.

8. Kuz'min, N. A., VETERINARIYA, 1968, p 2.

9. Levina, Ye. N., ZHMEI, No 1, 1958, p 9.

10. Levina, Ye. N., Gol'din, R. B., Noskov, F. S., Shakhonina, K. L., and Barskiy, V. Ye., "Lyuminestsiruyushchiye antitela v mikrobiologii" [Luminescent Antibodies in Microbiology], Moscow, 1972.

11. Maysel', M. N., Kabanova, Ye. A., Levina, Ye. N., and Pishchurina, M. M., IZV. AN SSSR. SERIYA "BIOLOGIYA", No 6, 1957, p 718.

12. Melikhov, A . D., "Sbornik nauchnykh trudov Estonskoy sel'skokhozyaystvennoy akademii" [Collection of the Scientific Works of the Estonian Agricultural Academy], Vol 4, 1958, p 106.

13. Meshcheryakov, A. Ya., "Trudy Vsesoyuznogo instituta eksperimental'noy veterinarii" [Works of the All-Union Institute of Experimental Veterinary Medicine], No 26, 1961, p 46.

14. Mikhaylov, B. Ya., Rozhkov, G. I., and Tamarin, A. L., ZHMEI, No 11, 1960, p 10.

15. Pritulin, P. I., and Kuz'min, N. A., VETERINARIYA, No 7, 1959, p 69.

16. Saltykov, R. A., "Spravochnik po mikrobiologii i virusologii" [Handbook of Microbiology and Virology], Moscow, 1967, p 282.

17. Shestochenko, M. A., VETERINARIYA, No 10, 1967, p 29.

18. Angelety, G. H., and Wright, G. G., APPL. MICROBIOL., Vol 21, No 1, 1971, p 157.

19. Mathois, H., MH. TIERHEILKUNDE, Vol 14, No 12, 1962, p 407.

20. Thorne, C. B., and Belton, F. C., J. GEN. MICROBIOL., Vol 12, No 2, 1957, p 505.

21. Cherry, W. B., and Freeman, E. M., ZBL. BAKT. I ABT. ORIG., No 175, 1959, p 582.

22. Dowle, W. R., and Hansen, P. A., J. INFECT. DIS., Vol 108, No 2, 1961, p 125.

PATHOGENESIS

Animals are infected by anthrax mainly via the alimentary pathway (Lincoln, Walerk, et al., 1964). Anthrax bacilli penetrate through injured mucous membrane covering different portions of the intestinal tract, getting into the lymphatic system and then the blood, where they are captured by phagocytes, and by which they are spread through the entire body. They are detained in elements of the macrophagal lymphoid system. A large quantity of bacilli reenter the blood, causing development of the diverse anthrax symptom complex, typified by intensive toxicosis.

An anthrax carbuncle--a focus of serous-hemorrhagic inflammation--may form when an animal is infected through the skin (by biting insects) (V. A. Vedernikov and S. I. Dzhupina, 1973).

Proliferating in the focus of inflammation, anthrax pathogen synthesizes and secretes exotoxin, causing general intoxication, and it penetrates very quickly into regional lymph nodes. Hemorrhagic lymphadenitis develops. The bacilli enter the blood, which spreads them throughout the body, in which they proliferate. Secondary septicemia sets in.

However, the carbuncular form of anthrax is rarely encountered. It may develop only among previously immunized animals. When they are infected by virulent anthrax bacillus strains, carbuncles appear only among animals whose unsusceptibility borders on sensitivity (V. I. Nikiforov, 1973). Usually the dose by which an animal is infected is too great, and massive edema and primary septicemia develop; the infecting dose might also be too low, in which case the typical infection process does not develop. The microbe's virulence and the body's overall immunological status doubtlessly have significance.

It is believed that animals die due to mechanical plugging of capillaries in vitally important organs, the lungs primarily, by bacilli. This hypothesis has never been experimentally confirmed (Stamatin, 1964).

Back in 1887 Toussaint believed that death is caused by anthrax due to depletion of blood oxygen. He explained this phenomenon by the fact that bacteria absorb all oxygen in the blood, causing asphyxiation.

Eckert and Bonventre (1963) also turned their attention to the extremely low blood oxygen concentration of animals in the terminal phase of the anthrax process. It was below the level commonly said to be critical.

However, Smith and Keppie (1962) do not share this point of view. They feel that acute anoxia observed in the terminal phase is an element of the shock syndrome, and that it cannot be recognized as the main cause of death.

Nor is great significance attached to hemolysis, since it sets in just prior to the animal's death, and in some cases it may even be absent entirely (Smith and Keppie, 1962).

Many aspects of the pathogenesis of anthrax infection were revealed after discovery of extracellular three-component toxin. Individual components are not very active, and it is only when they are combined that the toxin suppresses phagocytosis and causes tissue edema and death. Stamatin (1964) adheres to the same opinion. He points out that death of an animal due to anthrax results from serious physiological and anatomical disturbances arising in the body in the last stage of disease. These disturbances are elicited by a specific microbial factor--anthrax toxin, which causes the death of animals. Smith, Keppie, et al. (1965) feel that anthrax toxin may elicit secondary shock in the terminal phase of the infection process (about 8 hours prior to death), and that it may be the cause of death.

The injurious action of toxin upon the central nervous system, resulting in paralysis of the respiratory center, is given important significance in the pathogenesis of anthrax (Bonventre, Sueoka, et al., 1967; Klein, Lincoln, et al., 1968).

High vascular permeability and some loss of circulating fluid, noted upon introduction of toxin into the body, may have a certain significance to the pathogenesis of anthrax. Nevertheless this does not mean that the toxin directly affects vessels (Smith and Stoner, 1967).

Some researchers (Cromartie, Watson, et al., 1947; Bloom, McGhee, et al., 1947) have suggested the hypothesis that acute hyperglycemia is the cause of death in the presence of anthrax.

It has also been suggested that death due to anthrax is the result of disturbed calcium metabolism. A detailed study of biochemical processes demonstrated that in the very first hours after injection of toxin into experimental animals (white mice, rabbits), hyperglycemia arises and the activity of alkaline phosphatase increases (Slein, Logan, 1962). Adsorption of lethal toxin by cells of the reticuloendothelial system elicits an increase of their discharge of a number of other enzymes--serum aldolase, phosphoglucose isomerase, and glutamic and oxaloacetic acid transaminase. Production of these enzymes decreases when immune serum is introduced (Slein, Logan, 1960).

A study of the amino acid composition of blood serum from healthy and anthrax-infected guinea pigs (Smith and Tempest, 1957) established that in the terminal phase, anthrax bacillus makes use of a significant quantity of glutamine, threonine, tryptophan, and glycine. Histidine, lysine, and tyrosene are weakly utilized.

These observations attest to the possibility of competitive relationships between the parasite and the host in the struggle for vitally important metabolites during infection.

Thus animals die of anthrax as a result of profound disturbances in metabolism, which elicit secondary shock. The toxic complex produced by anthrax microbe has dominant significance to development of this complex biological process, and the course of disease depends to a significant extent on the quantity of the lethal factor present--the third component of the extracellular toxin produced by anthrax bacillus, the activity of which is stimulated by elective relationships with the edematogenic factor and protective antigen.

As was said earlier, the process of anthrax infection depends on the virulence of the agent and the organism's immunological expression.

Multiplying first at the place of its penetration, anthrax microbe in the susceptible organism surmounts the cellular and then the regional barriers (the lymph nodes) and penetrates deep into the body, eliciting septicemia (N. N. Ginsburg, 1966).

Regional lymph nodes partially detain the bacteria, and they are infected before the anthrax process becomes general.

Stamatin reports that only 9 percent of the spores are detained in the afferent lymphatic vessels leading to lymph nodes, while the rest (91 percent) are trapped by the lymph nodes. Vegetative forms of the microbe survive in even greater quantities in the lymph nodes, while not more than 0.03 percent of the introduced bacilli remain in the afferent vessels. However, anthrax bacilli are not lysed in the lymph nodes, where they may even undergo proliferation.

Proliferating in the body, vegetative cells synthesize capsule glutamine polypeptide and liberate toxin. The capsule substance inhibits opsonization, while the toxin elicits destruction of phagocytes, owing to which the body's most important protective function--phagocytosis--is disturbed (Keppie, Harris-Smith, and Smith, 1963). In addition anthrax toxin elicits edema and death of the animals.

Accumulation of toxin is completely dependent on an increase in the number of microorganisms in the blood. Keppie, Harris-Smith, and Smith (1955) published data indicating that 6 hours prior to death, 84 percent of the bacilli in guinea pigs are located in tissues, primarily the spleen. In deceased guinea pigs, 72 percent of the bacilli are detected in blood.

Penetrating out of the spleen, lymph nodes, and other organs into blood, the bacilli neutralize anthracocidal substances and undergo intensive reproduction. By the moment of the death of guinea pigs, there are up to $10^{8 \cdot 3}$ cells per milliliter of blood (Klein, Mahlandt, et al., 1960). A high concentration of microbes is also observed in the blood of animals of other species (Lincoln, Walker, et al., 1964). Thus animal death is preceded by developing septicemia.

In addition to the three-component exotoxin, pathogenic enzymes and microbial cell breakdown products apparently play a certain role in the pathogenesis of anthrax infection. Anthrax pathogen produces an extremely active proteolytic enzyme. Proteases produced by anthrax bacillus are exoenzymes. They may elicit breakdown of cellular proteins and enzymes in the sick organism, which could lead to the breakdown of tissues and metabolism.

hemorrhaging, dilation of lymphatic fissures and small lymph vessels, accumulation of anthrax pathogen, and its germination in the lumen of dilated lymphatic fissures and vessels.

As a result of germination of anthrax bacilli and absence of any mechanical obstacle to the lymph current on the part of lymph vessels, the microbes easily enter the bloodstream.

According to V. N. Nikiforov (1973) the throughput of lymph vessels is not disturbed in the presence of edema and necrosis. Owing to this, we always observe specific lymphadenitis, pronounced to the greatest extent in clusters of regional lymph nodes, where the bacteria are detained for a certain while; part of them perish, while the viable ones enter the bloodstream, causing the onset of septicemia.

Anthrax pathogen is liberated from the body of sick animals together with bronchial mucus, urine, and feces (S. Ya. Strelkov, 1924).

Anthrax bacilli may be detected in the milk and bile of sick animals (P. I. Kubasov, 1889; A. V. Kaymakov, 1939; V. M. Kovalev, 1959) as well as in urine and saliva (Sh. Kh. Kharisov, Kh. Kh. Abdullin, et al., 1971).

Isolation of anthrax bacillus from the organs of a fetus carried by a sick cow suffering subacute anthrax confirmed placental transfer of anthrax microbe in cattle.

Allergization of the body has been established in recent years in the pathology of anthrax infection. One of the constant signs of an allergic condition is a high quantity of eosinophils in blood. M. S. Yezhkova established that the quantity of eosinophils in artificially infected cows and goats attains 25 percent by the 14th day.

E. N. Shlyakhov (1960, 1964) proposed a preparation called antraksin for allergic diagnosis of anthrax. Only the allergizing factor of anthrax bacillus has not been established. Considering that cows immunized with STI vaccine do not react to an allergen or they react very weakly, and considering that cows artificially and naturally infected by epizootic strains of anthrax pathogen exhibit a very violent reaction, it should be presumed that the body is allergized by something other than exotoxin produced by this microbe (Kh. Kh. Abdullin, D. Sh. Akhmerov, Ts. Ts. Tsydypov). It may be presumed that capsule substance produced by the microbe or the products of its breakdown are what cause allergization of the body. Nor have we been able to clarify the distribution of this toxin in tissues and organs, the pathway by which toxin is liberated from the body, the mechanisms of detoxification, the pathogenic significance of the microbe's exo- and endoenzymes, and the anthracocidal factors of tissues and blood.

BIBLIOGRAPHY

1. Vedernikov, V. A., and Dzhupina, S. I., "Bolezni ovets i koz" [Diseases of Sheep and Goats], Moscow, 1973, p 158.

2. Ginsburg, N. N., "Rukovodstvo po mikrobiologii, klinike i epidemiologii infektsionnykh bolezney" [Handbook of the Microbiology, Clinical Pattern, and Epidemiology of Infectious Diseases], Moscow, 1966, p 293.

3. Kaymakov, P. V., "Trudy Kazanskogo nauchno-issledovatel'skogo in-ta" [Works of the Kazan' Scientific Research Institute], Kazan', Vol 5, 1939, p 71.

4. Kiselev, P. N., "Toksikologiya infektsionnykh protsessov" [Toxicology of Infectious Processes], 1971.

5. Kovalev, V. M., "Trudy Tsentral'nogo dezinfektsionnogo in-ta" [Works of the Central Disinfection Institute], Moscow, Vol 11, 1959, p 69.

6. Kubasov, P. I., VESTNIK OBSHCHEY GIGIENY SUDEBNOY I PRAKTICHESKOY MEDITSINY, No 1, 1889, p 1.

7. Nikiforov, V. N., "Kozhnaya forma sibirskoy yazvy cheloveka" [The Dermal Form of Human Anthrax], Moscow, 1973.

8. Stafanova, Ye. P., "Trudy Vsesoyuznogo in-ta eksperimental'noy veterinarii" [Works of the All-Union Institute of Experimental Veterinary Medicine], Moscow, Vol 22, 1959, p 177.

9. Kharisov, Sh. Kh., Abdullin, Kh. Kh., Krylov, Ya. A., and Levitskaya, M. N., "Aktual'nyye voprosy profilaktiki sibirskoy yazvy v SSSR" [Pressing Problems in Anthrax Prevention in the USSR], Moscow, 1971.

10. Shlyakhov, E. N., in "Epidemiologiya, diagnostika i profilaktika sibirskoy yazvy" [Epidemiology, Diagnosis, and Prevention of Anthrax], Kishinev, 1960.

11. Cromartie, W. J., Watson, A. W., Bloom, W. L., and Heckley, R. J., J. INFECT. DIS., Vol 80, No 1, 1947, p 14.

12. Keppie, J., Harris-Smith, P. W., and Smith, H., BRIT. J. EXPTL. PATHOL., Vol 44, No 4, 1963, p 446.

13. Lincoln, R. E., Walker, J. S., Klein, F., and Haines, B. W., in "Advances in Vet. Science," New York-London, Vol 9, 1964, p 327.

14. Slein, M. W., and Logan, G. F., J. BACTER., Vol 83, No 2, 1962, p 359.

15. Smith, H., and Tempest, D. W., J. GEM. MICROBIOL., Vol 17, No 3, 1957, p 731.

16. Smith, H., and Stoner, H. V., FEDERAT. PROC., Vol 26, No 5, 1967, p 1554.

17. Stamatin, N., J. MICROBIOL., PARASIT., EPIDEMIOL., Bucharest, 1964, p 477.

18. Stamatin, N., REV. IMMUNOL., Vol 32, No 1-2, 1968, p 67.

DISEASE SYMPTOMS AND COURSE

The symptoms and course of anthrax in agricultural animals depend on the pathway by which the infectious agent penetrates into the body, the species and individual sensitivity of the animal, the virulence of the microbe, and the point in time in the epizootic process.

The moment the organism comes in contact with the infectious agent is commonly adopted as the beginning of the disease's incubation period. It is rather difficult to determine this moment in relation to natural infection. It may be established for certain only with artificially infected animals. During the incubation period, toxin accumulates in tissues and the disease pathogen is liberated from the reticuloendothelial system; additional secondary foci of infection arise concurrently. Lincoln, Walker, Klein, and Haipes (1964) note that the body temperature of laboratory animals rises 24-36 hours after infection, which usually coincides with or follows initial bacteremia. K. N. Vinogradov (1874) noted an increase in temperature of colts infected with blood from a horse killed by anthrax 30 hours after infection, while the temperature increase occurred in a ram after 2 days. An increase in temperature was noted 32-40 hours after infection in experiments conducted by Kh. Kh. Abdullin, Sh. Kh. Kharisov, D. Sh. Akhmerov, and M. S. Yezhkova performed on goats infected with *Bac. anthracis* spores at doses of 5,000, 25,000, 50,000, and 100,000, introduced into the submucosa of the oral cavity and the root of the tongue. The incubation period in cows, once again infected through the submucosa but at large doses--5, 15, and 25 million and 1, 5, and 50 billion spores--lasted from 24 to 72 hours.

F. Gutira and I. Marek (1931) write that the length of the incubation period of anthrax varies from 1 to 14 days with artificial infection, and that it depends significantly on the place and intensity of the infection's development. Following subcutaneous and intravenous infection, the disease symptoms appear in as little as 24-48 hours. Sheep fed large quantities of spores usually die in 2-3 days; in natural infection conditions, however, when significantly fewer spores enter the intestinal channel, the incubation period is longer. Thus sheep fed hay sprayed with cultures containing anthrax spores in experiments conducted by Pasteur, Roux, and Chamberlaine did not fall ill until 10 days later.

A distinction is made between superacute (fulminant), acute, and subacute courses of the disease. Moreover the disease is classified in relation to the primary location of the infection process--dermal, intestinal, and pulmonary forms.

A superacute (fulminant) disease course is observed most often among sheep and cattle, usually at the beginning of an epizootic, and it is often characterized by cerebral apoplexy phenomena. The disease begins suddenly and proceeds severely. Animals exhibit convulsions, accelerated breathing, a fast weak pulse, cyanosis of visible mucous membranes, and loss of consciousness. The animal's gait is wobbly, it falls, and sometimes it bleeds from the mouth, the nose, and the anus. Death occurs in 30-60 minutes. Cases of an animal's sudden death during work, during grazing, or at night in a stall are observed.

A fulminant course represents the final, shock phase of disease. The animal's temperature reaction and the associated bacteremia phenomenon remain unnoticed.

Lincoln (1964) explains the death of the animal by the direct action of microbial toxin upon the central nervous system, resulting in paralysis of the respiratory center. Such a course of anthrax is typical of individuals that are especially sensitive to anthrax pathogen's toxin.

The acute course: Disease symptoms are observed over a longer period of time with this course of anthrax. The clinical signs are usually produced by cerebral hyperemia, in connection with which signs of restlessness dominate in stricken animals. At times the animal lies down, at other times it hops to its feet, it bellows, groans, gnashes its teeth, hurls itself against the manger, and beats the walls. This aroused state usually alternates with inhibition. Breathing becomes faster and heavier, heart sounds become loud, the pulse quickens and becomes barely tangible, and mucous membranes turn cyanotic. Spot hemorrhaging appears on the mucous membrane of the nasal cavity and especially the conjuctiva. Appetite is subdued. Milk secretion decreases or stops. The quality of milk also changes: It becomes yellowish or bloody-colored, its taste is bitter, and sometimes it has a mucous consistency. Pregnant animals may abort (I. Ravich, 1873; N. D. Stapanov, 1909; N. A. Mikhin, 1942; S. N. Vyshelesskiy, 1948; F. Gutira, I. Marek, 1954). Temperature rises to 40-42°.

N. N. Levitov (1931) and Lincoln (1964) note that a temperature decrease occurs in artificially infected rabbits and guinea pigs 2-4 hours prior to death. In the agonal state the contents of the intestine and the urine become bloody. Death occurs in the presence of asphyxia phenomena.

If edema does appear among sheep, cattle, and horses suffering this course of disease, it is extremely insignificant, and as a rule it is localized in the vicinity of the pharynx and larynx. N. D. Stepanov (1909) writes: "In our clinic there were several cases of anthrax among horses brought in with symptoms of acute edema of the larynx, developing on the background of anthrax infection."

A subacute course is encountered the most frequently, especially among horses. The disease symptoms are the same as with the acute course, but they are less violent. Disease persists for 7 days and more. At times of remission, which are exhibited to a greater degree among ruminants than among horses, the overall condition of the animal improves. It begins to take feed, and rumination appears. However, the apparent improvement is soon superseded by new attacks of fever. Edematous regions (carbuncles) appear on the skin with this disease course.

According to F. Gutir and I. Marek (1937), S. N. Vyshelesskiy (1945), and F. A. Terent'yev (1967) the abortive form of anthrax is observed among cattle. It is characterized by small rises in temperature and weakly pronounced clinical signs. Sometimes the disease lingers for up to 2-3 months, and as a rule the stricken animal becomes emaciated.

The carbuncular form: Disease accompanied by the appearance of edematous regions in dermal and subdermal tissues and in mucous membranes is defined as the carbuncular form of anthrax. It is encountered among horses and cattle. It lasts 5-7 days in cattle, and as a rule for a shorter time in horses. A distinction is made between the primary carbuncular form, in which a carbuncle appears at the place of introduction of the infection source, and the secondary form, where infiltrates form at different places on the body. Edema of the skin and subcutaneous tissue is noticeable around the arising carbuncle. Sometimes the carbuncle attains enormous proportions. At first it is sharply delimited, hard, and painless; then it deadens beginning at the center, and transforms into an ulcer. The edematous zone takes the form of a diffuse, flat, doughy, often fluctuating swelling. This form of disease is accompanied by an insignificant rise in body temperature lasting 3-7 days. Cases of recovery are observed.

A carbuncle appearing on mucuous membranes is diagnosed as anthrax of the tongue, pharynx, or throat. The disease is accompanied by high temperature, difficult swallowing and breathing, general cyanosis, and swellings at the entrance to the larynx and on the neck and dewlap. Death occurs after 12-14 hours (N. A. Mikhin, 1942).

V. F. Nagorskiy (1902) presents information on swelling location on the basis of A. P. Levitskiy's data on the frequency of occurrence of swelling in different parts of the animal body (Table 3).

The intestinal form manifests itself as a disturbance in the function of digestive organs. First constipation is observed, followed by diarrhea. The rectum often protrudes outward, and feces are eliminated with blood in the presence of very painful tenesmus. Signs of extremely severe cholic appear in horses.

In general the intestinal form of the disease is characterized by the most diverse symptoms, and it is accompanied by high temperature (N. A. Mikhin, 1942).

The pulmonary form of anthrax is encountered rarely among animals, and only in sheep (N. A. Mikhin, 1942). According to data cited by D. M. Teternik, N. G. Ipatenko, and others, it may also arise in pigs. With aerogenic infection, however, this form may develop among all animal species susceptible to anthrax, since it has been established that this method of infection is highly effective.

Disease symptoms observed among animals in response to experimental infection will be described below.

The research was conducted by Kh. Kh. Abdullin, Sh. Kh. Kharisov, D. Sh. Akhmerov, and M. S. Yezhkova in 1970-1971 on 37 animals: 24 milking goats from 1.5 to 10 years old with a live weight of 28-43 kg, and 13 milking cows from 3 to 13 years old, not vaccinated against anthrax. Clinical status was established prior to infection. In order to create more-natural infection conditions, the animals were

Table 3. Swelling Locations

Location	No. of Cases	Percent of Total
Horses		
On sex organs and the udder (600), in the groin (20), and simultaneously on other portions of the body (26)	646	55.1
On the chest (243) and simultaneously on other parts (16)	258	22.0
On the neck (99) and simultaneously on other parts (7)	106	9.1
On the pharynx	40	3.4
On the shoulders	26	2.2
On the abdominal walls	84	7.2
On other parts of the body (head, sides, legs)	12	1.0
	1,172	100.0
Cattle		
On sex organs and the udder (65), in the groin (22), and simultaneously on other portions of the body (8)	95	30.0
On the neck	69	21.9
On the pharynx	38	12.1
On the chest	35	11.1
On the shoulder	34	10.4
On the abdominal walls	23	7.0
On other parts of the body, including the sacrum (2)	31	7.5
	325	100.0

infected through the submucosa of the oral cavity and the root of the tongue. The disease course was acute and subacute in 17 out of 24 infected goats, abortive in 2, and latent in 5. It was acute and subacute in 8 cows, and abortive in 5.

Disease Symptoms in Goats

Acute course: Goat No 2. Twenty-four hours after infection the animal's overall condition was good. A swelling appeared on the left side of the upper lip after 32 hours. After 36 hours the temperature rose to 40.5°, an insignificant decline in overall condition became noticeable, pulse quickened to 110 beats per minute, appetite and rumination remained unchanged, and edema increased around the lips, the intermaxillary space, and head. A reduced milk yield was noted on the 2d day after infection.

Subacute course: Goat No 12. The goat was kept under observation for 134 hours after infection. During this time the animal's temperature and general condition did not change. An increase in pulse to 93 beats per minute was noted 48 hours after infection, but it did not exceed physiological limits in the subsequent period of analysis. The milk yield did not decline. The goat died on the 6th day.

Abortive course: Goat No 9. This goat was kept under observation for 186 hours after infection. During this time no visible clinical changes were detected. An increase in pulse to 98 beats per minute was noted after 64 hours, and an increase in temperature to 40.8° was noted after 88 hours. Breathing remained normal.

Disease Symptoms in Cows

Acute course: Cow No 6. Body temperature increased to 40.4° 22 hours after infection. Appetite and rumination remained unchanged. The cow's temperature climbed to 41.4° 38 hours after infection. Pulse and respiration increased. The general condition of the animal was subdued. The cow stood with its head lowered. The milk yield declined by 42 percent, and later the cow stopped giving any milk at all. Edema appeared in the intermaxillary space 36 hours after infection (Figure 22). On the 3d day the animal's temperature was 40.4°, its pulse was 72 beats per minute, and the frequency of breathing movements was 20 per minute. The cow's overall condition was depressed. It rejected feed. Edema spread to the neck area. The edematous zone was hard, painful, and hot to the touch. Body temperature dropped to 39° 64 hours after infection. Breathing became faster. The "ignition groove" [direct translation of "zapal'nyy zhelob"] was pronounced in the animal upon inspiration. Pulse frequency was 38 beats per minute. Overall condition deteriorated. Appetite and rumination were absent. Urination and defecation increased in frequency. The urine was dark red. Convulsions and swimming motions of the limbs appeared. The animal died 70 hours after infection.

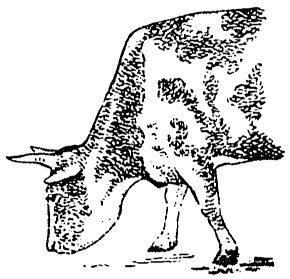

Figure 22. Edema of the Intermaxillary Space of a Sick Cow 36 Hours After Infection

Subacute course: Cow No 1. An increase in body temperature to 40.6° was noted 76 hours after infection. Pulse and respiration frequency were within normal. Appetite and rumination persisted. Temperature increased to 41.2° after 88 hours, while pulse and respiration remained without special changes. Edema appeared in the intermaxillary space. Appetite and rumination persisted. General condition was satisfactory. Temperature was within 40-40.8° 96-152 hours after infection. During this time, edema increased, filling the intermaxillary space and spreading to the upper part of the neck. On the 3d day the edematous region assumed a firm consistency and turned cold. Overall condition was subdued. Appetite and rumination were absent. The milk yield declined. On the 10th day, temperature dropped to 39.6°. Edema abated somewhat. In the evening bloody discharges appeared from the cow's vagina, and general condition worsened. In the evening on the 11th day after infection bloody discharges once again appeared from the vagina, and the cow aborted.

On the 12th-15th days edema decreased in the intermaxillary space and disappeared from the neck region. Temperature, pulse, and respiration returned to normal, and rumination resumed. On the 15th day the cow was killed for further analysis. Post-mortem bacteriological analysis of the cow's organs in BEA revealed luxuriant growth of typical anthrax microbe colonies.

Abortive course: Cow No 5. Twenty hours after infection the animal's general condition deteriorated, body temperature increased to 41.2°, and appetite and rumination persisted. Pulse and respiration were within normal. After 44 hours body temperature dropped to 39.8°, and general condition worsened. After 104 hours the animal's temperature and general condition returned to normal. Two days after infection the milk yield dropped by 65 percent, remaining at this level until the end of the experiment. The experiment lasted 9 days.

Chronic course: Cow No 9. A rise in body temperature to 40.2° was noted 23 hours after infection. Temperature climbed to 40.8° 32 hours after infection. The cow's general condition was good. Appetite and rumination persisted. Pulse frequency did not exceed 60 beats per minute. Body temperature dropped to 39° after 72 hours. However, it climbed to 41.8° by the 80th hour. Pulse and respiration remained normal at this time. General condition was subdued, and appetite persisted. Temperature attained 42.2° after 96 and 104 hours. Pulse (80 beats per minute) and respiration (44 breathing movements per minute) increased in frequency. General condition was subdued, and appetite was absent. Temperature dropped to 38.7° 158 hours after infection. Pulse and respiration returned to normal. An urge for food appeared. General condition improved. Edema in the intermaxillary space disappeared 8 days after it appeared.

On the 3d day after infection the daily milk yield was 65 percent. It dropped to 25 percent on the 8th day. In subsequent days (up to the 60th) the milk yield fluctuated within 20-50 percent.

BIBLIOGRAPHY

1. Vyshelesskiy, S. N. "Chastnaya epizootologiya" [Specific Epizootiology], Moscow, Sel'khozgiz, 1948.

2. Gutira and Marek, "Chastnaya patologiya i terapiya zhivotnykh. Infektsionnyye bolezni" [Specific Animal Pathology and Therapy. Infectious Diseases], 1931.

3. Mikhin, M. N., "Sibirskaya yazva cheloveka i sel'skokhozyaystvennykh zhivotnykh" (Anthrax in Man and Agricultural Animals], Moscow, 1942.

4. Nagorskiy, V. F., "Arkhiv veterinarnoy nauki" [Veterinary Science Archives], Book VII, 1902.

5. Ravich, I., "Arkhiv veterinarnoy nauki," Book I, 1873.

6. Stepanov, N. D., "Kratkiy kurs infektsionnykh bolezney" [A Short Course on Infectious Diseases], Kazan', 1909.

7. Terent'yev, F. A., TRUDY VIEV, Vol 34, 1967.

8. Lincoln, R. E., Walker, J. S., Klein, F., and Haines, B. W., "Advances in Veter. Science," 1964, p 9.

PATHOMORPHOLOGY AND PATHOMORPHOLOGICAL DIAGNOSIS

Pathoanatomical changes occurring in the presence of anthrax depend on the nature of the disease course and the location of the pathological process. The following forms of anthrax are commonly distinguished: septic, carbuncular (dermal), intestinal, and pulmonary.

The septic form: The following characteristics attract attention upon autopsy of animals dying with clinical symptoms of the septic form of disease: absence of rigor mortis (or it is weakly pronounced), swelling, discharge of foamy bloody fluid and sometimes dark blood from natural openings, cyanosis of visible mucous membranes, and multiple spot and pinpoint hemorrhaging on the conjuctiva and on mucous membranes of the nasal and oral cavities and the anus; serous or serous-hemorrhagic infiltrates coupled with hemorrhaging in subcutaneous and intermuscular tissue, beneath the costal pleura, in the mediastinum, in perirenal fat, and in other places. Muscles are flabby, dark red, and pinpoint and spot hemorrhaging is present on and in muscles. The spleen is highly enlarged (sometimes by several orders of magnitude), its margins are rounded, the pulp is dark red or black, it is highly softened, and it has a pasty consistency. In severe forms of disease the pulp drains from the surface of an incision as a tarry mass. The capsule is highly tensed, and it is ruptured here and there. Lymph nodes, both superficial and ones in the internal organs, are highly enlarged, swollen, dark red or reddish black, moist upon dissection, riddled with points of hemorrhaging, and discharging bloody fluid in response to compression; surrounding tissues are saturated with serous-hemorrhagic transudate.

Severe lesions can almost always be found in the gastrointestinal tract, especially in the duodenum and jejunum. The intestinal mucosa is swollen, dark red, and covered by numerous areas of point and spot hemorrhaging, and Peyer's patches and solitary folliculi are eroded. Its surface is necrotic and ulcerated in individual areas or in its entirety in the presence of severe injury. The serous membrane is grayish red, and it contains bulging blood-filled vessels. The mesentery is in a state of serous edema (filled with gelatinous infiltrates) with numerous points of minor hemorrhaging. Lymph vessels in the mesentery appear as red strands oriented in the direction of regional lymph nodes. Alterations of the latter are similar to those described above in other lymph nodes.

The liver is enlarged, swollen, pale gray or grayish brown, flabby, the surface of an incision is dull, the pattern is smoothed, and there are numerous points of hemorrhaging in the parenchyma. Alterations of the same nature can be seen in the kidneys and heart. The lungs exhibit peristatic hyperemia and phenomena of serous edema. The mucous membrane of the larynx, trachea, and bronchi is hyperemic, it is riddled with numerous points of hemorrhaging, and it is edemic.

There are accumulations of bloody fluid in the pericardium and in the thoracic and abdominal cavities. The brain and spinal cord are hyperemic. Areas of pinpoint and spot hemorrhaging are present in both brain matter and brain membranes. There are transudate accumulations in the brain ventricles. Blood is uncoagulated, and it is dark red or reddish black in color (having the consistency of tar or varnish). It oxidizes slowly in air, acquiring a ruby color. The carcasses of animals dying from anthrax decompose quickly.

Histological analysis reveals the following: in the spleen--leukocytosis, hemorrhagic infiltration (invasion) by erythrocytes, necrosis and disintegration of the pulp, and serous-hemorrhagic edema of trabeculi and the capsule. In the lymph nodes--hemorrhagic inflammation: Vessels in sinuses are highly dilated, and filled with erythrocytes. The latter accumulate in medullary cords and follicles (as points of hemorrhaging). Small quantities of leukocytes, and sometimes fibrin fibers as well, are detected among erythrocytes. A typical pattern of hemorrhagic or hemorrhagic-necrotic enteritis is present in the intestine, while grainy dystrophy is evident in the liver, kidneys, and heart.

The carbuncular (dermal) form typically manifests itself in man. It is typified by development of focal, sharply pronounced hyperemia and inflammatory infiltration of the skin. The latter swells considerably at the center of the afflicted area in the form of a cone with a blunt tip. Epithelium covering the upper part of the cone sloughs off, and a fluid-filled blister forms. The fluid is transparent at first, then it becomes cloudy, and finally it turns dark red. This is followed by necrosis, and together with surrounding tissue the blister dries out and transforms into a dark brown or black scab. Subcutaneous tissue in the region of the afflicted skin is in a state of serous or serous-hemorrhagic edema, which spreads extensively into the surrounding area.

The dermal form of anthrax coupled with formation of primary carbuncles is rarely encountered in animals (mainly in pigs, horses, and rabbits). It usually occurs in combination with other forms, and it is typified by development of restricted areas of swelling subjected to necrosis and ulceration at the center. Primary pustules filled with bloody fluid sometimes arise. Serous-hemorrhagic infiltration of subcutaneous tissue also develops in afflicted areas.

The intestinal form is characterized by development of hemorrhagic inflammation in the small intestine. The duodenum and jejunum are afflicted predominantly. In some cases the process is focal, while in others it is diffuse, being accompanied by hemorrhagic inflammation of lymph nodes and lymph vessels in the mesentery (regional vessels) and discharge of hemorrhagic transudate into the abdominal cavity.

The most severe changes accompanying focal affliction occur in solitary follicles and Peyer's patches. They are the sites of inflammatory and necrotic processes. Autopsy reveals them to be rounded or longitudinally oval elevations of dark red or reddish black color, frequently covered with fibrinous films--carbuncles. Later they undergo necrosis and transform into grayish red or grayish brown scabs.

Histological analysis in the early stages of the process reveals serous-hemorrhagic inflammation of the intestinal wall, which is especially pronounced in the mucosa and submucosa, and infiltration of villi by polymorphic leukocytes. Leukocyte accumulations are also found in the submucosa. The mucosa and submucosa are necrotic, and formation of a diphtheric scab is noticeable in places (Figure 23).

Local lymph and blood vessels exhibit hemorrhagic lymphangitis, angitis, and thrombuses and, with a prolonged course, necrosis. Thrombolymphangitis and thromboangitis are also observed in remote vessels of the mesentery.

Histological changes accompanying the pulmonary form of disease have not been studied adequately. Autopsy usually reveals hemorrhagic or serous-hemorrhagic pneumonia, sometimes complicated by pleuritis identical in nature to hemorrhagic exudation into the pleural cavity. Bronchial lymph nodes may also be afflicted; hemorrhagic lymphadenitis of varying degrees may develop in them (Figure 24).

Figure 23. Anthrax Carbuncle in a Cow's Intestine

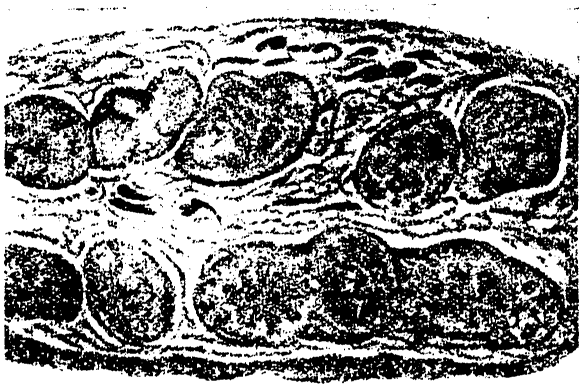

Figure 24. Hemorrhagic Necrotic Lymphadenitis in a Pig

In the anginal (tonsillar) form of anthrax, which occurs mainly in pigs, the epithelium of the tonsils is the first to be afflicted. Dystrophic, necrobiotic, and necrotic changes develop within it, owing to which diphtheric scabs form on the surface of the tonsils, beneath which foci of hemorrhagic inflammation arise, accompanied by progressive necrosis of the entire depth of tonsillar tissue. Necrotic areas are wedge-shaped, and their incision surface is swollen, being grayish red in early stages. These areas are surrounded by a peripheral zone of hyperemia and edema.

Incision of necrotic areas in the presence of chronic disease reveals them to be grayish yellow or grayish brown, dry, and smooth.

The inflammatory process often spreads to the larynx, epiglottis, soft palate, and subcutaneous tissue in the vicinity of the larynx, neck, head, and sometimes the dewlap. In such cases the cellular tissue of these areas appears as gelatinous infiltrates of pale or dark red color. Sometimes (in acute disease) edema of the tongue and palate are observed. Carbuncles are visible on the tongue, which subsequently undergo ulceration. A pattern of serous-hemorrhagic or necrotic lymphadenitis together with all signs typical of it is observed in local lymph nodes (retropharyngeal, submaxillary, more rarely upper cervical).

The apoplectic form of anthrax is specially distinguished by the nature of pathological development. In this form, pathoanatomical changes are located mainly in the brain. They express themselves as extreme plethora of vessels (especially veins) in the cerebral membranes.

Serous-hemorrhagic leptomeningitis often develops. In such cases sections of the afflicted pia mater appear swollen, thickened, and dull, with areas of solitary or multiple pinpoint and spot hemorrhaging. In some cases hemorrhaging is observed in subdural and subarachnoid spaces, as well as in the brain ventricles and in brain matter.

In view of swift death, changes do not occur in internal organs, or they are very weakly pronounced and are not entirely typical of anthrax.

The most typical signs in this case are: peristatic hyperemia of subcutaneous tissue, skeletal musculature, the liver, lungs, serous membranes of the intestine, and the peritoneum. Gelatinous infiltrates may sometimes be seen in certain areas of subcutaneous and retroperitoneal tissue. Points of minor hemorrhaging are also encountered in the peritoneum and pleura.

In addition to disturbed hemodynamics in organs, histological analysis reveals dystrophic alterations of gangliar cells in the brain (mainly in the cerebral cortex). When hemorrhaging is present (predominantly perivascular), polymorphic leukocytes and a small quantity of lymphocytes are found among erythrocytes.

So-called atypical forms of anthrax are also known (mainly among cattle and horses). Limited injury to particular organs occurs without development of a septic process in the body as a whole. The locations of these forms is the most diverse. As an example examination of cattle and horse carcasses following slaughter sometimes reveals serous-hemorrhagic infiltration of subcutaneous tissue in the vicinity of the mandible; hemorrhagic inflammation of the pharynx coupled with encapsulated necroses

in the tonsils and hemorrhagic inflammation of epiglottal and submaxillary lymph nodes; hemorrhagic enteritis accompanied by hemorrhagic lymphadenitis of mesenteric lymph nodes. In some cases scarred carbuncular ulcers are seen in the intestine together with affliction of mesenteric lymph nodes.

In addition miliary foci of necrosis surrounded by a peripheral zone of hyperemia have been described in the parenchyma of the adrenal cortex in cattle, while focal fibrinous pleuritis is observed in horses. Restricted areas of serous-hemorrhagic inflammation of the skin coupled with hemorrhagic-necrotic lymphadenitis of corresponding local lymph nodes are also encountered.

Anthrax proceeds differently in agricultural animals of different species. The septic form of anthrax is observed as a rule in cattle, sheep, and horses. However, cases are known in which carbuncular afflictions of varying location (depending on the place of introduction of the infectious agent) develop in them on the background of septicemia. This is usually seen in cattle and horses. The apoplectic form of disease is often recorded among sheep.

Autopsy of fur-bearing animals (usually mink and raccoon) killed by anthrax reveals pathoanatomical ulcerations similar to those seen with the septic or intestinal form of anthrax in domesticated animals: peristatic hyperemia and hemorrhaging in the mucous membranes, serous coverings, and in the parenchyma of organs; considerable enlargement of the spleen coupled with softening of the pulp; enlargement of lymph nodes (especially epiglottal and cervical) coupled with hemorrhaging and serous edema of surrounding tissues; enlargement of the adrenal glands; degeneration of the liver, kidney, myocardium, and skeletal muscles; pulmonary edema. The most serious afflictions are observed in the gastrointestinal tract. In the stomach--intense hyperemia and hemorrhaging of the mucous membrane; in the small intestine (mainly the duodenum)--considerable swelling and disintegration of the mucous membrane, and multiple hemorrhaging, in addition to hyperemia.

Histological analysis reveals: catarrhal-hemorrhagic gastroenteritis; serous-hemorrhagic lymphadenitis and splenitis, accompanied by necrosis and inhibition of the reticuloendothelium.

Pathomorphogenesis: The primary focus forms at the place of penetration and proliferation of anthrax bacilli. Its location varies depending upon the infection pathways: skin, nasal pharynx (tonsils), small intestine. It is characterized morphologically by development of serous-hermorrhagic inflammation, preceded by a defensive cellular proliferative reaction. Later the causative agent passes through lymph vessels to the corresponding local lymph nodes, in which serous-hemorrhagic or hemorrhagic lymphadenitis develops.

Experimental data confirm that in the early stages of infectious development, anthrax microbes spread by way of the lymphatic system. Thus infection of rabbits and guinea pigs with an anthrax culture (Trnka, Malek, Sterzi, and Kolek, 1958) revealed that 4 hours after subcutaneous and cutaneous injection of spores into the animals, blood cultures produced negative results. Later, microbes were found in lymph and local lymph nodes and, in subsequent stages, in the spleen and lungs. They were detected in blood in the final stages of infectious development. The authors also established that after different doses of spores are introduced into lymph nodes, more animals die and death occurs more quickly than with subcutaneous infection, even when smaller doses are employed.

Using a permanent lymphatic-venous anastomosis to study the dynamics behind the spread of microbes to the lymphatic system, the authors demonstrated appearance of microbes in lymph 10-16 hours sooner than in the blood of all animals they studied.

Under certain conditions the agent may pass through local protective barriers without damaging them, attacking only the local lymph nodes. Affliction of epiglottal lymph nodes in the absence of alterations in the pharynx and tonsils, or affliction of mesenteric nodes without formation of a carbuncle in the intestine may serve as an example in pigs. The primary focus may be present in the body for a long period of time, seemingly in a resting state; this is observed, as an example, in cattle, pigs, and horses.

Diagnosis: As we know, the bacteriological method is the main way to establish an anthrax diagnosis, autopsy of the carcass and pathomorphological analysis being an auxiliary and forced method. Autopsy of carcasses is permitted only in exceptional cases, particularly during forensic veterinary expert examination. This is explained by the danger of spreading anthrax bacilli in the environment. In cases where the need arises for performing an autopsy on the carcass of an animal killed by anthrax, the appropriate sanitary and hygienic rules are complied with strictly.

Anthrax should be differentiated from some acutely proceeding infectious diseases at the time of the autopsy: in cattle and sheep--from the acute form of pasteurellosis, malignant edema, emphysematous carbunculosis, and babesiosis; in horses--from infectious anemia and babesiosis; in pigs--from plague, erysipelas, hemorrhagic septisemia, and intoxications. The acute (thoracic) form of pasteurellosis in cattle is distinguished from the septic form of anthrax by presence of fibrinous necrotic (catarrhal-fibrinous in young animals) pneumonia, often coupled with serous-fibrinous pleuritis, and more rarely coupled with pericarditis and peritonitis. The principal signs of edemic pasteurellosis include extensive serous edema of subcutaneous and intermuscular tissue of the head, neck, and dewlap, pronounced especially intensively in the vicinity of the pharynx and the intermaxillary space (gelatinous infiltrates). Acute serous lymphadenitis and nonhemorrhagic lymphadenitis is observed in the presence of pasteurellosis (coupled with affliction of predominantly the bronchial and mediastinal lymph nodes). Septic changes are absent from the spleen.

Infectious anemia in horses (the acute form) differs from the septic form of anthrax by the following basic signs: pallor and, frequently, jaundice of visible mucous membranes coupled with multiple spot hemorrhaging, observed most often in the conjuctiva of the nictitating membrane and the mucous membrane of the lips, jaundiced coloration of subcutaneous tissue (in addition to serous infiltration and hemorrhaging), and enlargement of the spleen without considerable softening of the pulp. Blood is watery and light red.

Babesiosis in horses (nuttaliosis) and cattle differs from the septic form of anthrax in that the former is accompanied by more sharply pronounced jaundice of mucous membranes and serous coverings, by serous edema (gelatinous infiltration) of subcutaneous tissue without hemorrhaging, by milder enlargement of the spleen and no softening of the pulp, and by absence of diffuse or focal serous hemorrhagic inflammation of the gastrointestinal tract.

In all cases where difficulties arise in differential diagnosis of anthrax from these diseases during autopsy and histological analysis, the final decision should be based on the results of bacterioscopic and bacteriological analyses.

BIBLIOGRAPHY

1. Al'meyev, Kh. Sh., "Uchenyye zapiski Kazanskogo zoovetinstituta" [Scientific Notes of the Kazan' Zooveterinary Institute], Vol 49, 1938, p 2.

2. Vyshelesskiy, S. N., "Infektsionnyye i invazionnyye bolezni loshadey" [Infectious and Invasive Diseases of Horses], Moscow, 1948, p 123.

3. Ivanov, B. G., and Nikolayevskiy, L. D., SOVETSKOYE OLENEVODSTVO, No 7, 1936, p 85.

4. Mikhin, N. A., "Sibirskaya yazva cheloveka i sel'skokhozyaystvennykh zhivotnykh" [Anthrax in Man and Agricultural Animals], 1942.

5. Nikiforov, V. N., "Problemy zoonozov" [Problems of Zoonosis], Stavropol', 1964, p 148.

6. Terent'yev, F. A., Kosarev, V. A., and Terent'yev, A. F., TRUDY VIEV, Vol 34, 1967, p 312.

7. Tkachenko, A. F., TRUDY KAZANSKOGO VETINSTITUTA, No 2, 1936, p 85.

8. Teternik, D. M., SOVETSKAYA VETERINARIYA, No 4, 1936, p 20.

9. Lamb, R., BRIT. MED. J., No 1, 1973, p 157.

10. Marek, P., VET. REC., Vol 81, No 16, 1967, p 418.

11. Ramachandran, S., and Waterajan, M., INDIAN VETER. J., Vol 45, No 5, 1968, p 381.

12. Stamatin, N., J. MICROBIOL., PARASITOL., EPIDEMIOL., Bucharest, Vol 9, No 6, 1964, p 477.

13. Trnka, Z., Malek, P., Sterzl, J., and Kolc, J., CS. MIKROBIOL., Vol 3, No 2, 1958, p 82.

THE BLOOD PICTURE OF ANIMALS STRICKEN WITH ANTHRAX

The very first researchers to discover anthrax microbes in animals stricken and killed by this disease observed changes in the blood picture. Thus, studying blood from sheep killed by anthrax, Rayer (1850) and Pollender (1855) noted a tendency for erythrocytes to stick together in clumps of indefinite form.

N. F. Kolesnikov (1889, 1891) also found morphologically altered erythrocytes in horses stricken by anthrax. He reported the discovery of erythrocytes without concavities, and enlarged, cloudy, swollen erythrocytes that did not stick together like stacks of coins. V. F. Nagorskiy (1871) also noted the last phenomenon.

It has been noted that a decrease occurs in erythrocyte count both in response to infection of animals by a virulent culture of the pathogen and as a result of injection of vaccine strains. This phenomenon was observed by many authors in animals of different species: by I. M. Sadovskiy (1885, 1888), V. V. Kolokolov (1893), and R. A. Kadymov and N. M. Ibragimov (1968) in sheep, by N. F. Kolesnikov in horses, by Yu. P. Kvitkin (1961) in reindeer, and by V. V. Kolokolov and A. V. Koronnyy (1958) in rabbits. The maximum decrease in erythrocyte count was noted on the 2d through the 4th days after administration of vaccine strains to the animals. On the 8th day the erythrocyte count returns to normal. The number of erythrocytes decreased to 1 million cells in reindeer in response to vaccination (Yu. P. Kvitkin), while in rabbits experiencing an infection process it dropped to as much as half the normal amount (A. V. Koronnyy). A. V. Vasil'yev (1948) noted that toward the end of the period of disease, the erythrocyte count decreases from 8 million to 5 million.

Degenerative and regenerative processes also occur in the blood of sick animals: anisocytosis, poikilocytosis, hypochromic polychromatophilia, and even a normoblastic blood picture (A. V. Koronnyy); nucleated erythrocytes appear (V. V. Kolokolov).

A decrease in hemoglobin is observed concurrently with a decrease in the erythrocyte count of vaccinated and infected animals (N. F. Kolesnikov, N. Ryazantsev, 1891; R. A. Kadymov and N. M. Ibragimov; Yu. P. Kvitkin).

Most authors noted an increase in the leukocyte count in the presence of anthrax (I. N. Lange, 1871; I. M. Sadovskiy; I. I. Mechnikov, 1885; N. F. Kolesnikov; V. V. Kolokolov, 1893). V. V. Kolokolov reported that not long before death, the leukocyte count increases by 1.5-3 times in comparison with normal; sometimes he observed a decrease in the number of leukocytes.

A. V. Kornnyy observed an insignificant increase in leukocyte count, an increase in the number of neutrophils coupled with an insignificant leftward nuclear shift in the leukocyte formula, and a decrease in the lymphocyte count in experimentally infected rabbits.

A. V. Vasil'yev reported an abrupt increase in the leukocyte count (hyperleukocytosis) in response to anthrax. In some vaccinated animals the leukocyte count attained 22,000, while in animals contracting the disease naturally it increased to 50,000. If the condition of the sick animal deteriorates, the leukocyte count drops dramatically. R. A. Kadymov and N. M. Ibragimov observed a similar pattern in infected sheep. G. V. Cheglokova (1960), who infected animals with 20-50-time doses of Tsenkovskiy vaccine No 2, observed an increase in the quantity of leukocytes to 12,500-14,300, while in normal conditions their number did not exceed 8,500.

Immunizing reindeer with STI vaccine, Yu. P. Kvitkin noted an increase in leukocytes to 7,000 on the 2d day (4,500 being normal). Their quantity returned to normal on the 8th day.

A. V. Vasil'yev noted lymphocytosis coupled with a concurrent decrease in neutrophil count in the first days of illness. Neutrophilia is observed subsequently. The concentration of neutrophils increases to 75 percent in vaccinated animals and to 85 percent in naturally stricken animals. According to R. A. Kadymov and N. M. Ibragimov an increase in the neutrophil count is observed in infected sheep at the beginning of disease; later this condition is superseded by an increase in lymphocytes and monocytes. A. V. Vasil'yev notes that the leukocyte formula of neutrophils undergoes a nuclear shift to younger forms if the disease is mild, and to myelocytes in severe cases.

Performing experiments on 12-month sheep and female lambs, G. I. Romanov (1973) also observed growth in the leukocyte count following injection of STI vaccine and a virulent culture of anthrax pathogen into the animals. One or two days after vaccination the leukocyte count increased from 12,600-16,600 to 24,600-27,500, while after infection with the virulent culture the leukocyte count increased to 40,000 in some animals.

Other changes in blood characteristics are observed as well. For example the sedimentation rate increases on the 3d day after vaccination of sheep (Yu. P. Kvitkin), and the concentration of oxygen, carbon dioxide, and nitrogen decreases (N. Ryazantsev). The quantity of calcium increases in vaccinated sheep, reindeer, and rabbits (M. F. Smetkin and S. M. Gol'berg, 1928; A. B. Boyakhchyan and Ye. G. Paroyan, 1951; Yu. P. Kvitkin). The calcium concentration drops dramatically just prior to death (A. B. Boyakhchyan, Ye. G. Paroyan).

In the first days after vaccination the concentration of histamine increases in animals, and it is not until the 3d day that it begins to decline. The activity of the enzyme histaminase increases as well (A. B. Boyakhchyan and M. S. Grigoryan, 1952).

R. A. Kadymov and N. M. Ibragimov studied the activity of phagocytes in sheep, and they established that it depends on the outcome of infection. In animals that survived, phagocyte activity increased without a subsequent decline, while in animals that died, the phagocyte activity increased for a short period of time, and then declined.

It was in adult animals that the authors observed the described changes in the blood picture.

Experiments performed to study changes in the blood picture of the young of small farm animals following vaccination with STI anthrax vaccine were performed by G. I. Romanov (1971) on Tsygay lambs 2, 3, and 4 months old, obtained from ewes that had and had not been vaccinated against anthrax.

There were eight vaccinated lambs in each age group (four lambs from vaccinated ewes, and four lambs from unvaccinated ewes). The animals were given subcutaneous injections of dry STI anthrax vaccine at a dose of 8 million microbial bodies. The injection was made on the inner surface of the thigh. Twenty-four healthy unvaccinated lambs of corresponding age and origin served as the control.

Blood was sampled from the auricular vein of the animals, in the morning on an empty stomach, before vaccination (to establish a normal level) and after 6 and 12 hours and 1, 2, 3, 5, 7, 10, and 15 days.

The erythrocyte and leukocyte counts, the concentration of hemoglobin per milliliter of blood, and change in the leukocyte formula were determined. Nuclear shifts in the leukocyte formula of the lambs were assessed on the basis of the nuclear index of the neutrophils, which was determined with the formula

$$\frac{M+Ю+П}{M+Ю+П+С},$$

proposed by Sh. D. Moshkovskiy. The experimental results were said to be positive when the significance level was below 0.05 (95 percent).

The results of hematological analyses revealed that the blood picture of healthy lambs differs somewhat from the blood picture of mature sheep. Hypoeosinophilia is observed in the blood of lambs; in 2-month lambs it is accompanied by neutrophilia, which disappears at 3 months of age.

In addition the blood of lambs 2 and 3 months old, and especially of the former, containsimmature forms of neutrophils. This causes a leftward shift in the nuclear index. Immature neutrophils are not encountered as a rule in 4-month sheep. The nuclear index of neutrophils in 2-months lambs was 0.12, that of 3-month lambs was 0.13, and that of 4-month lambs was 0.07.

Injection of STI anthrax vaccine into experimental lambs produced a responding reaction on the part of hemopoietic organs. A significant change in the leukocyte count and the hemoglobin concentration did not occur in 2-month lambs after vaccination. But the erythrocyte count decreased on the 2d, 3d, 5th, and 7th days after vaccination from 11.77 ± 0.4 million per ml (normal) to 10.51 ± 0.4 million, 10.26 ± 0.5 million, 10.84 ± 0.3 million, and 9.91 ± 0.2 million per ml respectively.

A significant increase in the nuclear index of neutrophils was observed in lambs vaccinated at an age of 2 months. Thus in lambs obtained from vaccinated ewes, this index increased from 0.08 ± 0.01 (normal) to 0.19 ± 0.01 a day later, attaining a maximum (0.267 ± 0.1) on the 3d after immunization. An increase in the nuclear index of neutrophils in lambs of this group was noted 1, 2, 3, 7, and 15 days (the period of observation) after vaccination.

The neutrophil index increased to a lesser degree among vaccinated lambs obtained from unvaccinated ewes. A significant change in hematological characteristics was not noted among unvaccinated lambs throughout the entire period of the research.

A significant change in the erythrocyte and leukocyte counts was noted among 3-month lambs after vaccination. Thus the erythrocyte counts decreased from 11.32 ± 0.3 million per ml (normal) to 9.95 ± 0.32 million on the 3d day after immunization, while among unvaccinated lambs of the same age a significant decrease in the erythrocyte count was not observed at these times. The leukocyte count of the lambs increased from 9,600 ± 600 per ml in normal conditions to 11,660 ± 400 and 11,150 ± 400 per ml on the 2d and 3d days after vaccination respectively, while among unvaccinated lambs an increase in the leukocyte count was not detected at these times. Significant change was not revealed in the hemoglobin concentration of vaccinated and unvaccinated lambs of this age group.

The neutrophil nuclear index increased significantly in vaccinated lambs obtained both from vaccinated and from unvaccinated ewes, while changes did not occur in the nuclear index of neutrophils in control animals.

A significant increase in leukocyte count was noted after vaccination of lambs 4 months old--from 10,400 ± 500 per ml in normal conditions to 11,400 ± 400, 11,300 ± 600, and 10,300 ± 400 per ml on the 1st, 2d, and 5th days respectively. An insignificant decrease in the leukocyte count occurred at these times in unvaccinated lambs--from 10,500 ± 400 per ml in normal conditions to 9,500 ± 400, 9,400 ± 400, and 8,600 ± 300. While the leukocyte count of unvaccinated lambs decreased, among vaccinated animals it increased or remained at the same level. Thus the differences in the figures for immunized and control animals were significant at these times.

The nuclear index of vaccinated lambs from both vaccinated and unvaccinated ewes increased significantly.

Significant change in erythrocyte count and hemoglobin content was not noted in vaccinated lambs of this age in comparison with unvaccinated control animals throughout the entire period of research.

Thus injection of STI anthrax vaccine into lambs 2, 3, and 4 months old elicits a responding reaction on the part of hemopoietic organs, characterized in lambs 3 and 4 months old by an increase in the quantity of leukocytes in peripheral blood. A decrease in the erythrocyte count is noted after vaccination of lambs 2 and 3 months old.

A leftward shift of the nuclear index of neutrophils occurs following vaccination of lambs 2, 3, and 4 months old; immature forms of neutrophils, such as juvenile neutrophils and myelocytes, are observed in the blood of the animals.

The leukocyte count experiences a two-time increase after vaccination of 4-month lambs; this is true of adult sheep as well; the leukocyte count experiences only a one-time increase in 3-month lambs, while for 2-month lambs the increase in the leukocyte count is insignificant. This circumstance as well as other changes in the blood picture permit the hypothesis that 4-month lambs respond to a fuller extent to injection of STI vaccine than do younger lambs (especially 2-month lambs), and that animals of this age group exhibit reactivity similar to that of adult sheep.

This hypothesis is also supported by the results of acute experiments, described in the chapter on immunity, performed to test immunity generated by lambs of different ages in response to injection of STI vaccine.

Thus these data demonstrate that when both virulent and vaccine anthrax strains are introduced into the animal body, the changes in the blood picture of the animals are typified by a decrease in the quantity of erythrocytes, hemoglobin, oxygen, carbon dioxide, and nitrogen, and an increase in the quantity of leukocytes, calcium, and histamine. The erythrocyte sedimentation reaction usually quickens.

BIBLIOGRAPHY

1. Boyakhchyan, A. B., and Paroyan, Ye. G., TRUDY YEREVANSKOGO ZOOVETERINARNOGO INSTITUTA, No 13, 1951, p 247.

2. Boyakhchyan, A. B., and Grigoryan, M. S., TRUDY YEREVANSKOGO ZOOVETERINARNOGO INSTITUTA, No 14, 1952, p 41.

3. Vasil'yev, A. V. "Gematologiya sel'skokhozyaystvennykh zhivotnykh" [Hematology of Agricultural Animals], Moscow, 1948.

4. Kvitkin, Yu. P., "Sbornik nauchnykh rabot. (Saratovskaya nauchno-issled. vet. stantsiya)" [Collection of Scientific Works (Saratov Veterinary Scientific Research Station)], Saratov, No 5, 1961, p 25.

5. Kolesnikov, N. F., AVN, No 5, 1889, p 65.

6. Kolokolov, V. V., VESTNIK OBSHCHESTVENNOY VETERINARII, 1893, p 19.

7. Koronnyy, A. V., "Tezisy dokladov ob"yedinennoy nauchnoy konferentsii, posvyashchennoy izucheniyu Voronezhskoy oblasti" [Abstracts of Reports of the Combined Scientific Conference Devoted to Study of Voronezhskaya Oblast], Voronezh, 1955, p 27.

8. Lange, I. N., AVN, No 2, 1871, p 106.

9. Mechnikov, I. I., VETERINARNYY VESTNIK, Vol 1, No 2, 1885, p 31.

10. Nagorskiy, V. F., AVN, No 3, 1871, p 227.

11. Romanov, G. I., TRUDY GNKI, No 20, 1974, p 206.

12. Ryazantsev, N., "Sbornik trudov Khar'kovskogo veterinarnogo instituta" [Collection of Works of the Khar'kov Veterinary Institute], No 3, 1891, p 545.

13. Sadovskiy, I. M., AVN, No 4, 1885, p 316.

14. Smetkin, M. F., and Gol'berg, S. M., "Uchenyye zapiski Kazanskogo vetinstituta" [Scientific Notes of the Kazan' Veterinary Institute], Vol 38, No 2, 1928, p 77.

15. Rayer, P. F., O. C. R. SOC. BIOL., No 2, 1850, p 141.

16. Pollender, F. A. A., VIERT. F. GERICHTK. U. OFF MED. BERLIN., No 8, 1855, p 103.

TREATMENT OF SICK ANIMALS

Between the time that anthrax became known to mankind and the present, the treatment of sick animals experienced many changes, and it has as long a history as that of the disease itself.

As recently as at the beginning of the past century sick animals were treated by bleeding, and carbuncles were lanced and seared with red-hot iron. Animals were given Creolin solutions of various concentrations internally. Carbuncular infiltrate was removed with solutions of carbolic and salicylic acid.

After the disease pathogen was discovered, more-radical treatment methods appeared. Thus Mosedonio (1882) used ammonium hydroxide, and the results were not bad. Jolly (1882) tested the action of iodine, concluding that its intravenous injection into cattle at doses from 9 to 15 gm, depending on animal age, often produces a positive result.

Naturally most of these methods and therapeutic resources are no longer used today, being only of historic interest.

Antianthrax Serum

Treatment of animals stricken by anthrax was advanced by the introduction of anti-anthrax serum. It was obtained in 1895 by Sclavo in Italy and Marchu in France, independently of one another. Later many researchers worked to improve it. Our domestic scientists contributed an especially great deal of effort to obtain highly active serum.

Antianthrax serum was obtained for the first time in Russia by Yurgelyunas (1902). In 1906 N. A. Pokshishevskiy obtained active serum from a sheep and a horse, and in 1908 from two horses. Although at the beginning of his work the author set the goal of achieving hyperimmunization as quickly as possible, he needed 7 months to acquire active serum.

In 1908-1910 S. N. Vyshelesskiy managed to obtain active antianthrax serum from horses in 51 days. F. I. Bautts (1910) and Kuleshov (1911) also obtained active serum by hyperimmunization of horses with a quick method.

In 1911 N. A. Mikhin conducted experiments on accelerated hyperimmunization of several horses, obtaining active serum in 55 days.

P. I. Borovskiy (1912), N. A. Pokshishevskiy and S. K. Bezzubets (1926), G. A. Kudryavtsev and D. S. Romanov (1926), S. A. Alekseyev (1930), and others subsequently obtained active antianthrax serum.

Beginning in the 1930's, it took biological plants about 3 months to prepare horses; spore cultures of virulent anthrax microbes were used for hyperimmunization. A method was developed and proposed in 1939 for obtaining active antianthrax serum in a short time by hyperimmunization of horses with a virulent vegetative anthrax culture to which alum is added as the precipitating agent (S. G. Kolesov). This method is used today at biological plants to acquire antianthrax serum broadly employed for both preventive and therapeutic purposes.

Antianthrax serum is obtained from hyperimmune horses. Animals from 3 to 6 years old obtained from farms free of infectious diseases are used as the producers. To obtain active serum, the horses are first subjected to massive immunization with the purpose of creating background immunity. For this purpose they are given four doses of Tsenkovskiy No 2 vaccine and STI vaccine, administered alternately as subcutaneous and intracutaneous injections over a period of 2 weeks.

The animals are first hyperimmunized subcutaneously and intracutaneously, and then, beginning with a dose of 10 ml, only subcutaneously with increasing doses of a virulent day-old broth culture of anthrax pathogen, increasing the total volume of the culture to 160-180 ml. A mixture consisting of four virulent strains is injected at one time. In all, 12 strains are used for hyperimmunization, each administered in succession. The strain suspension is first injected without alum. Beginning with the 10 ml dose, 0.2 percent alum is added to the culture. After 80 ml are administered, 0.1 percent alum is added.

The general condition and temperature of the producers is constantly monitored during hyperimmunization, and if the temperature rises, the next injection is given only after it drops again. The entire process of hyperimmunization takes about 9 weeks.

Ten days after hyperimmunization, blood is sampled from each producer to test the titer of the serum's activity.

Serum is acquired by citration of blood with sodium citrate, followed by separation and defibrination of the plasma. Each series of serum is tested for sterility by culturing it in nutrient mediums, and for harmlessness by injecting it subcutaneously into five white mice at a dose of 0.5 ml, and into two guinea pigs at a dose of 10 ml each.

Cultures grown on nutrient mediums (BEA, BEB, and BELB [beef-extract liver broth]) should be sterile, and all animals should remain alive. Serum activity is tested as follows: Five rabbits weighing 2-2.5 kg are given intravenous or subcutaneous injections of the serum at a dosage of 2 ml per kilogram animal weight. Then a virulent culture of anthrax pathogen is injected subcutaneously into the same rabbits. In the first method serum is injected simultaneously with virulent culture, and in the second it is injected 24 hours before infection. Three rabbits are used as controls. The serum is said to be active if a minimum of three experimental rabbits survive while all control animals die.

Mass use of antianthrax serum in practical conditions has demonstrated its effectiveness.

Serum is injected subcutaneously in the following doses for preventive purposes: horses, adult cattle, and deer--15-20 ml; sheep, goats, calves, and pigs--8-10 ml. Unsusceptibility to anthrax persists in animals for up to 15 days following serum injection.

Serum is used for therapeutic purposes when anthrax is diagnosed. It is injected subcutaneously into sick animals in the following doses: horses, camels, adult cattle, and deer--from 100 to 200 ml; sheep, goats, and pigs--from 50 to 100 ml. To avoid anaphylactic phenomena in all animals except horses, and when repeat injections are to be given in 10-14 days, serum is first injected at a dose of 0.3-0.5 ml for small animals and 1-2 ml for large animals; the complete dose is injected 15-30 minutes later. Injection of more than 30 ml serum in the same place is not recommended. If the disease course is severe, serum first heated to 37-38°C is injected intravenously as the therapeutic dose. When necessary, it may be administered again after 5-6 hours. The best results are achieved when it is given to animals in the early stage of disease.

Antianthrax Gamma-Globulin

Antianthrax globulin has been used extensively in medical practice in the last 10 years. Biological industry produces it not only for medical but also veterinary purposes.

Antianthrax globulin is the gamma-beta-globulin fraction obtained from antianthrax serum by precipitation with ethanol at low temperature. The method was developed by M. A. Babich and V. A. Plotnikova (1961). The preparation is made as a solution containing 10 percent protein, and it is repacked in 20-ml flasks. Its shelf life is 2 years from the moment of manufacture when stored at 4-15°C.

During production, antianthrax gamma-globulin is tested for its protein and alcohol concentration, pH, physical properties, purity of the protein fraction, sterility, harmlessness, and activity. Dissolved gamma-globulin must be transparent or slightly opalescent, it must be colorless, and it should not contain suspended particles or sediments. It would be desirable for the pH of the solution to be from 7.0 to 7.4. The purity of the gamma-globulin fraction in relation to protein fraction impurities is tested by paper electrophoresis. The concentration of this fraction in solution must be not less than 90 percent.

Gamma-globulin is tested for sterility by inoculation on BEA, BEB, and BELB beneath petrolatum oil. All cultures held at 37°C for 10 days must be absolutely sterile.

Gamma-globulin is tested for harmlessness by subcutaneous injection into five white mice weighing 15-17 gm (0.5 ml dose), and two guinea pigs weighing 350-400 gm (10 ml dose). All animals must remain alive for 10 days.

The activity of each series is tested on rabbits. Six animals weighing 2-2.5 kg are given intravenous injections of gamma-globulin at a dosage of 1 ml per kg animal weight. These animals and three control animals are simultaneously infected subcutaneously by a virulent culture of anthrax pathogen. All control animals must die, and not less than four of the experimental animals must survive.

Antianthrax gamma-globulin is used for preventive and therapeutic purposes. It is infected subcutaneously into the animals at the following doses: for preventive purposes, 3-4 ml for horses, adult cattle, and pigs; for therapeutic purposes, 40-80 ml for horses, camels, adult cattle, and deer, and 20-40 ml for sheep, goats, calves, and pigs.

Animals retain unsusceptibility to anthrax for up to 14 days following injection of gamma-globulin for preventive purposes.

The preparation is used for therapeutic purposes when an anthrax diagnosis is established. If the disease course is severe, the preparation is first heated to 37-38°, and then it is injected intravenously at the therapeutic dose. If the condition of the sick animal does not improve after 5-6 hours, globulin is reinjected at the same dose.

As with the use of antianthrax serum, the best therapeutic effect is achieved with antianthrax gamma-globulin when it is introduced in the early stage of disease.

Use of Antibiotics and Medicinal Agents

Carbolic and salicylic acid solutions were used extensively to treat animals stricken with anthrax prior to acquisition of antianthrax serum. These solutions were injected subcutaneously or administered internally, and sometimes the results were favorable.

In 1811 Becker (L. A. Rozen'yer, 1948) proposed salvarsan therapy for treatment of people stricken with anthrax. After this a large number of reports appeared confirming Becker's results. This preparation also came into use in veterinary practice. However, Sterne (1959) found that this preparation is ineffective in experimental infection, and he stated his preference for neosalvarsan. The latter was also used extensively in medical and veterinary practice prior to discovery of sulfamide preparations and antibiotics. Discovery of the latter and establishment of the relatively high sensitivity of anthrax pathogen to them led to extensive use of these preparations.

Antibiotics came into use in the treatment of animals stricken with anthrax in the late 1940's and early 1950's.

The sensitivity of anthrax bacilli to penicillin, aureomycin, tetracycline, streptomycin, and levomycetin was established by Garrod (1952). Boger (1953) classifies antibiotics in the following order of decreasing effectiveness in the treatment of animals: aureomycin, terramycin, levomycetin, penicillin, streptomycin, and neomycin. G. P. Belikov et al. (1956) established that penicillin saved the lives of more than half of the mice they infected with an anthrax culture, synthomycin saved the lives of one-fourth of them, and biomycin treatment saved all mice.

Stern (1959) believes that penicillin is the most active; however, allergic reactions may be observed sometimes with its use, and it is ineffective against local afflictions. Penicillin probably is not able to neutralize the tissue-injuring factor which, as we know, is associated with infection of the body by anthrax bacilli.

Jones, Klein, Lincoln, et al. (1967) established through *in vitro* experiments that out of dehydrostreptomycin, chloramphenicol (levomycetin), chlorotetracycline (biomycin), and penicillin, chlorotetracycline is the most active. The same authors noted that penicillin and dehydrostreptomycin were the most effective against anthrax in mice (penicillin was administered at a dose of 1,000 units, and the other preparations were given at a dose of 0.4 mg each. Later, penicillin was used at a dose of 200 units, while the other preparations were used at doses of 2 mg each).

Sterne reported that according to data cited by Goret, Collet, Peris, and Gourouble (1948), penicillin at a dose of about 2 million units per day cured anthrax in cattle exhibiting signs of bacteremia.

Beyli (Ya. Ye. Kolyakov, 1965) reported successful use of procaine-penicillin and terramycin. He used these preparations to treat 208 head of cattle and six horses, and he achieved good results (97.4 percent of the animals recovered when treated with penicillin for 3 days). Greenough (1965) treated eight cows of one farm with penicillin and streptomycin; seven of the animals recovered as a result of the treatment (5 million units of crystalline penicillin were administered three times a day for 3 days; the streptomycin doses are not indicated). The author concluded that treatment effectiveness depended on the time treatment was started and the period over which the preparation was used, and on this basis he recommended treating sick animals for a minimum of 3 days; he also believed that treatment success depended on early diagnosis of disease. Lincoln (1964) and Cold (1967) also advised using streptomycin combined with penicillin to treat anthrax in animals. The preparations are administered intravenously at a dose of 1-2 gm for 14 days.

Spears and Davidson (1959) recommend antibiotics (penicillin, chlorotetracycline) in rapidly assimilable forms in the initial stage of treatment of anthrax in animals, so as to create a high concentration of these antibiotics in the blood faster. Penicillin was also found to be very effective at a dose of 10,000 units per kg animal weight. Treatment must continue for several days after temperature normalizes.

G. V. Cheglokova (1960) experimentally studied the therapeutic effectiveness of penicillin, streptomycin, and biomycin. She infected three kids 2.5 months old with 20-50-time doses of Tsenkovskiy No 2 vaccine; simultaneously she injected penicillin in camphor oil into one kid, streptomycin into the second, and a mixture of these preparations in to the third (1,000 units of each preparation in five injections), and she was unable to elicit illness in the animals. The fourth control kid died 60 hours after infection.

Antibiotics were administered to colts, lambs, and sheep when clinical signs of disease appeared. Streptomycin was injected into two colts 12 months old: The first dose was 800,000 units, and subsequent doses were 500,000 each. One colt was given four injections, and the other was given eleven. Three lambs 3 months old were given a mixture of bicillin and streptomycin. Bicillin was injected once at a dose of 200,000 units, and then streptomycin was injected at a dose of 100,000 units twice a day for 5 days until recovery. Two sheep received a single bicillin injection of 300,000 units, followed by four streptomycin injections of 200,000 units each. All animals recovered.

These data attest to the good therapeutic effect of various antibiotics in the presence of anthrax. We also share this point of view, but we believe that better

results may be achieved through joint prescription of antibiotics and serotherapy. This opinion is shared by Lincoln, Walker, Klein, Heines, Sterne, G. P. Rudnev, A. I. Kolobkova, and N. G. Ipatenko.

Biomycin and terramycin can also be used. From 8 to 18 gm biomycin and terramycin are recommended for the entire course of treatment, which usually lasts up to 10 days. In the first 3 or 4 days of disease these antibiotics are used at doses from 0.5 to 0.75 gm at 4-hour intervals, and after condition improves, a dose of 0.5 gm is given at 6-hour intervals. The effectiveness of these preparations is approximately the same.

In addition to the drugs indicated above, symptomatic agents should be prescribed for sick animals: glucose intravenously, and caffeine, camphor oil, and other preparations subcutaneously. They are administered in therapeutic doses.

Thus it should be presumed that the best results may be achieved in treating anthrax in animals with antibiotics combined with antianthrax serum or antianthrax globulin.

BIBLIOGRAPHY

1. Vsevolodov, V. I., "Opyt ucheniya o poval'nykh boleznyakh mezhdu domashnimi zhivotnymi" [Experience With the Teaching on Epidemic Diseases Transmitted Among Domesticated Animals], St. Petersburg, 1846.

2. Khotovitskiy, S., "O sibirskoy yazve" [On Anthrax], St. Petersburg, 1831.

3. Geyrot, F., "Medikoprakticheskoye opisaniye o bolezni tak nazyvayemoy sibirskoyu" [A Practical Medical Description of the Disease Named Siberian], St. Petersburg, 1807.

4. Rozen'yer, L. A., "Sibirskaya yazva u cheloveka" [Anthrax in Man], Kishinev, 1948.

5. Belikov, G. P., et al., ZHMEI, 1956, p 106.

6. Kolyakov, Ya. Ye., "Veterinarnaya mikrobiologiya" [Veterinary Microbiology], Moscow, 1965.

7. Cheglokova, G. V., VETERINARIYA, No 7, 1960, p 43.

8. Sobernheim, G., "Milzbrand. Handbuch der Pathogenen Mikroorganismen," 1929.

9. Boger, W. P., ARCH. DERMATHOL. A. SYPHILIDOL., Vol 67, 1953, p 541.

10. Davis, E., Hidgman, S. F., and Skulski, G., VETERINARY RECORD., Vol 69, No 33, 1957, p 775.

11. Garrod, L. P., ANTIBIOT. CHEMOTHER., No 2, 1952, p 689.

12. Greenough, P. R., VETERINARY RECORD., Vol 87, No 27, 1965, p 784.

13. Jones, W., Klein, F., Lincoln, R., et al., JOURNAL OF BACTERICLOGY, Vol 94, No 3, 1967, p 609.

14. Sterne, M., "Infectious Diseases of Animals," London, 1959.

IMMUNITY

Animals of different species are known to be variably susceptible to anthrax. Even animals of the same species maintained in equal conditions are not all infected with anthrax. In the first case we refer to specific unsusceptibility to anthrax, in the second to individual resistance of susceptible animals.

The reasons for the anthrax resistance exhibited by animals of some species and the resistance of certain animals of the same species have not been clarified yet. Only theoretical suggestions may be stated on this issue for the moment.

It has been noted that animals surviving anthrax become unsusceptible to repeat infection. They develop so-called postinfectional immunity.

Postvaccinational immunity also forms as a result of immunization of animals with anthrax vaccines containing live spores weakened by subjecting the microbes to heat, or unencapsulated mutant microbes selected from virulent strains. The degree of unsusceptibility to anthrax exhibited by vaccinated animals depends on their reactivity and on the physiological state of the body at the time of vaccination.

A Short History of the Vaccine's Acquisition

Mankind came up with the idea of creating preparations that could make the body unsusceptible to infectious diseases very long ago, but it was not until the end of the 18th century that it could be realized. This was the time when smallpox vaccine was discovered. Eighty-five years later the prominent French scientist Louis Pasteur managed to conquer another disease--anthrax. He and his colleagues made a vaccine against this disease in 1881.

Immunization against anthrax was first attempted in 1880. These attempts were made independently of one another by Chauveau and Toussaint.

Chauveau demonstrated that unsusceptibility to anthrax could be achieved after vaccination. Observing a certain degree of inborn unsusceptibility to this disease among Algerian sheep, he concluded that introduction of small quantities of blood containing anthrax pathogen into sheep would intensify their unsusceptibility even more, and create resistance against infection by a virulent anthrax culture.

Toussaint established that sheep do not get anthrax in response to administration of a virulent pathogen culture, if they are previously given a subcutaneous injection

of defibrinated blood containing bacilli, heated for 10 minutes at 55° or passed through filter paper prior to injection. He also indicated a second means for obtaining vaccine--addition of a small quantity of carbolic acid to blood containing the disease pathogen. He demonstrated this immunization method to a commission of the Alfort Veterinary School. The experiment was not entirely successful: Of 20 vaccinated animals, 4 died after immunization, in view of which this principle did not enjoy application (A. Arkhangel'skiy, 1884).

Louis Pasteur preferred another way for obtaining anthrax vaccine, which he had used earlier to weaken fowl cholera (pasteurellosis) pathogen. But it was discovered that rather than weakening when being exposed to atmospheric air, anthrax microbes form spores.

In 1881 Pasteur reported that he had developed a method for acquiring anthrax vaccine consisting of the following. A virulent culture isolated from the carcass of an animal killed by anthrax is inoculated into chicken broth and placed in a thermostat at 42-43° for attenuation.

Chicken infection experiments Pasteur performed led him to the idea that high temperature must be used to weaken the virulence of anthrax microbes. Infection of chickens with a virulent culture did not cause disease because the body temperature of chickens is higher than that for optimum growth of anthrax pathogen. However, by reducing the body temperature of chickens (by placing them in cold water), Pasteur was able to infect birds and cause them to die of anthrax. Pasteur proved in this experiment that to reduce the virulence of anthrax microbe, it must be inoculated in nutrient broth and grown at a higher temperature in a thermostat, in the presence of atmospheric oxygen. Inasmuch as spore formation does not occur at 42-43°C, this temperature causes a gradual decrease in the virulence of anthrax bacilli.

Holding a culture of anthrax microbes in a thermostat at 42-43° for 24 days, Pasteur obtained one vaccine weakened to a certain degree, while holding a culture for 10-12 days he obtained a second vaccine weakened to a lesser degree. To stabilize the degree to which the resulting anthrax vaccines were weakened, he subjected the bacillar form of the microbes to spore cultivation in a thermostat at 35°.

After subjecting the obtained vaccines to laboratory analysis to test their residual virulence in laboratory animals (white mice, guinea pigs, and rabbits), in summer 1881 Pasteur performed a publicized experiment at the farm of (Puyile For) to test the vaccine. Animals (24 sheep, 1 goat, and 6 cows) were immunized on 5 May with the first vaccine and on 17 May with the second.

On 31 May all vaccinated sheep, the goat, and cows were infected with virulent anthrax pathogen culture. None of the animals fell ill. Unvaccinated sheep and goats died in 2 days; the cows remained alive, but they were extremely sick. The second experimental infection of vaccinated sheep was performed 8 months after immunization, once against producing positive results. These experiments made an extraordinary impression upon the entire world.

In that same year, 1881, Louis Pasteur and his assistants vaccinated 70,000 head of cattle against anthrax, and next year they vaccinated 400,000. Pasteur animal immunizations began to be performed in many other European countries, and a favorable assessment was given to the vaccine. This elicited tremendous interest among scientists, practical veterinarians, and livestock breeders in many countries.

This discovery served as a great stimulus for many leading scientists interested in developing the methods of active prevention of infectious diseases in both medical and veterinary practice.

Anthrax also caused severe losses in animal husbandry in Russia, especially in the country's southern regions. When the existence of effective antianthrax vaccine became known, the question of visiting Pasteur's laboratory to gain an acquaintance with the methods of the vaccine's acquisition was raised. L. S. Tsenkovskiy, a professor of Khar'kov University, was sent to Pasteur's laboratory in Paris. In 1882, after returning from France, L. S. Tsenkovskiy began work on anthrax vaccines. In 1883 he obtained anthrax vaccine of two degrees of attenuation by subjecting a virulent culture of anthrax pathogen to high temperature. He seeded the virulent anthrax culture in chicken broth and grew it in a thermostat at 42.5° for 12 days to obtain vaccine No 1, and for 6 days to acquire vaccine No 2. In order to make the resulting properties of the bacillar form of vaccine microbe constant, L. S. Tsenkovskiy induced spore formation by growing the microbe for 6 days at 35°.

L. S. Tsenkovskiy first tested the obtained vaccines on white mice, susliks, and rabbits. Persuading himself that the virulence of the vaccines in relation to laboratory animals had declined, he began testing them on sheep. Positive results were achieved in the very first experiment, performed on a small number of sheep (11 vaccinated and 2 control).

In 1884 L. S. Tsenkovskiy and his assistants--I. M. Sadovskiy, G. L. Skadovskiy, and V. Voznessenskiy--performed a major experiment to test anthrax vaccines obtained with sheep. The experiment was performed at Belozerka Manor in the presence of a commission consisting of veterinarians, physicians, and other representatives of the Kherson provincial zemstvo.

In this experiment, performed 15-18 November, 1,333 sheep were immunized first by vaccine No 1 and then by vaccine No 2. Of these, 30 vaccinated sheep were infected with a virulent anthrax pathogen culture. Unvaccinated sheep were infected concurrently. All 30 vaccinated sheep remained alive, and 9 of the 10 control sheep died of anthrax. Soon after this, the vaccines began to be used on other animals as well.

L. S. Tsenkovskiy noted that spore vaccines grown in broth were unstable, in view of which different vaccine series were heterogeneous. In order to stabilize the vaccines and lengthen their shelf life, Tsenkovskiy proposed preparing them by confining broth spore cultures in 30 percent glycerin solution

In 1887 a commission composed of council members, veterinarians, and agronomists and appointed by the Kherson provincial zemstvo conducted an experiment to test the immunity of sheep 13 months following immunization with Tsenkovskiy vaccines No 1 and No 2. Three out of 20 immunized sheep died, while 9 out of 10 control sheep died. Such a commission was also created in 1890. Both commissions concluded that these vaccines successfully protect sheep against anthrax.

A similar vaccine was obtained in 1890 by I. N. Lange, a professor of the Kazan' Veterinary Institute.

So-called French vaccines produced by a laboratory in Nizhegorod came into use in Russia beginning in 1895.

In 1897 the Ministry of Internal Affairs appointed a commission to compare Tsenkovskiy's, Lange's, and the "French" vaccines. The commission did its work in the Don and Saratov provinces, and it concluded that in comparison with the others, Tsenkovskiy's vaccines produced stabler and longer-lasting immunity.

Many researchers tried to prepare vaccine against anthrax. However, none of the proposed vaccines came into practical use because they did not exhibit pronounced immunogenic properties.

In the form in which they were proposed by their inventors, live vaccines such as Pasteur's and Tsenkovskiy's had a number of shortcomings. They included, in particular, imperfections in the method for making the vaccines (the vaccines were prepared by cultivation in broth), and their instability during use. It is for this reason that many researchers subjected both the vaccination method and the vaccines themselves to improvement. By as early as 1896-1897 Tsenkovskiy's bacillar vaccines were replaced in Russia by their spore forms.

In 1902 Sobernheim proposed a combined (simultaneous) immunization method: Antianthrax serum was administered simultaneously with a culture having somewhat attenuated virulence, corresponding approximately to that of Pasteur's vaccine No 2. The results were favorable. The simultaneous method is less dangerous, but it is more expensive and painstaking, and less effective than ordinary anthrax vaccines.

In 1911 S. N. Vyshelesskiy reported efforts to improve the procedure for growing Tsenkovskiy anthrax vaccines. He demonstrated the possibility for using beef-extract agar in place of chicken broth to grow the vaccines, which significantly improved their quality. In the time of this work at the veterinary-bacteriological laboratory of the Ministry of Internal Affairs (1906-1914), S. N. Vyshelesskiy demonstrated that preventive vaccination of animals was the principal way to fight anthrax.

In the 1920's A. M. Bezredka proposed intracutaneous vaccination. It was used in a number of countries, including ours. However, despite the positive results, it did not enjoy extensive practical use.

In 1929 Mazzuchi proposed adding 2-5 percent saponin to virulent or slightly attenuated strains. Such processing produced an active safe vaccine. But undesirable reactions to saponin and the risk of using insufficiently attenuated strains in this method made it necessary to prepare this vaccine from weakened strains.

Hruska (1931-1933) reported successful use of saponin-vaccine in Czechoslovakia.

F. A. Terent'yev proposed glucoside vaccine (saponin-vaccine) in the Soviet Union in 1934. It was Tsenkovskiy vaccine No 2 spores in saponin solution. It was used for a number of years, but although it possessed pronounced immunogenic properties, it did not always produce constant values of biological activity because saponin used to prepare the vaccine was often nonstandard, being highly toxic and causing the death of a significant quantity of spores in the vaccine.

Practicing veterinarians observed that Tsenkovskiy vaccine No 1 did not always prepare animals adquately for administration of vaccine No 2, andthat immunization with vaccine No 2 without preliminary vaccination produced stable immunity. This observation served as the grounds for studying the possibility of vaccinating animals with

vaccine No 2 alone. Work by F. A. Terent'yev, A. G. Rebnivykh, I. S. Startsev, I. Ya. Ogarev, V. Novikova, and others demonstrated the possibility for using Tsenkovskiy vaccine No 2 alone to immunize agricultural animals.

Pasteur's, Tsenkovskiy's, and some other anthrax vaccines were known to possess significant shortcomings: They had to be administered twice, and sometimes they elicited severe postvaccinational complications. The reasons for this were, on one hand, the condition of the animal's body at the time of immunization and, on the other hand, the biological features of these vaccines. These vaccines are known to consist of microbes having residual virulence in relation to laboratory animals (white mice, guinea pigs), and they cause pronounced sepsis and death of such animals. The explanation given for this by some researchers (N. N. Ginsburg, S. G. Kolesov, Stamatin, Sterne) is that microbes in Pasteur's and Tsenkovskiy's vaccines have capsulogenic properties, which are responsible for significant residual virulence in relation to laboratory animals. Therefore in order to obtain improved vaccines against anthrax, scientists assumed a new path--isolating and selecting genetically altered capsule-less variants of anthrax pathogen. Microbes of such variants are typified by complete loss of the ability to form a capsule in the animal body and in artificial nutrient mediums, absence of the capability for eliciting sepsis when introduced into white mice and guinea pigs, and high immunogenic properties.

Bail noted back in 1915 that when anthrax pathogen is grown in serum medium at higher temperature, some individuals form without capsules. Following 25-fold subinoculation in this medium, such individuals lose their ability to form a capsule not only in nutrient mediums but also in the mouse body.

Studying variability of anthrax microbes grown in coagulated defibrinated or citrated horse blood, Stamatin (1934) established the possibility for obtaining weakly virulent edematogenic capsule-less strains. One of them (1190-R) formed flat, round colonies in beef-extract agar, and it was used to manufacture vaccine. According to a report by N. Stamatin and L. Stamatin (1936) this vaccine was highly immunogenic in experiments with mice, rabbits, and sheep. It was first used in Romania on sheep in 1938, and it has been used to immunize all animals since 1950 (Stamatin and Isopescu, 1957).

Sterne (1937) obtained the capsule-less immunogenic variant 34 F_2 on 50 percent serum agar in a CO_2 atmosphere (65 percent). This variant was proposed for preparation of anthrax vaccine. Vaccine made from strain 34 F_2 is used today for preventive immunization of animals against anthrax in many countries.

Inoculating coagulated normal equine serum in Petri dishes, in 1940 N. N. Ginsburg of the USSR obtained the vaccine capsule-less mutant STI-1 (Sanitary-Technical Institute) from a virulent culture of "Krasnaya Niva" strain. Tests on laboratory and large animals demonstrated its harmlessness and immunogenecity. Vaccine prepared from this strain came into use in our country beginning in 1942. It proved itself well as a highly immunogenic preparation.

Using a method of alternating inoculation of virulent strain Shuya-2, isolated in 1933 from a pig carcass, on coagulated normal equine serum and peptone-free agar, in 1946-1949 S. G. Kolesov obtained the weakly virulent strain Shuya-15, which was found to be harmless and immunogenic in tests on laboratory animals. In 1951-1952 S. G. Kolesov and Yu. F. Borisovich prepared GNKI vaccine from this strain by adding

glycerin and aluminum hydroxide; tests of this vaccine on sheep showed it to be highly immunogenic. In 1953-1955 the vaccine was tested and introduced into practice by S. G. Kolesov and N. A. Mikhaylov. It has been used extensively since 1961 for immunizations in dry form (without glycerin and aluminum hydroxide).

Thus vaccines prepared from attenuated strains obtained as a result of their exposure to temperature factors were replaced by vaccines prepared from strains having the typical features of hereditary loss of the ability to form capsules and, consequently, of causing disease in animals, inasmuch as the pathogenicity of anthrax agent is associated with the capsule. STI and GNKI anthrax vaccines are now being made and successfully used in the Soviet Union.

Associated Vaccines

As early as in 1926, the French scientist Ramon used associated vaccination against anthrax and tetanus, anthrax and smallpox, and other diseases, thus demonstrating the possibility for using such vaccines in the practice of animal immunization.

Delpi (1952) reported the possibility of immunizing animals with associated vaccine against anthrax and emphysematous carbuncles, and against anthrax and smallpox in sheep.

Attempts at obtaining associated vaccines against anthrax and other diseases and at developing methods of simultaneous vaccination began in our country in the 1930's, but they became especially widespread in recent years. This problem has been worked on by F. I. Kagan and Ya. R. Kovalenko (1932), M. D. Polykovskiy and A. N. Burdov (1962), A. B. Boyakhchyan and P. L. Kalashyan (1967), S. G. Kolesov and F. I. Kagan, A. I. Kolesova, L. P. Rudenko, G. I. Romanov, and L. I. Storozhev (1969), A. V. Selivanov and V. V. Marinkova (1973), and others.

Efforts to create associated vaccines are still continuing today.

Colleagues of the Kazakh SSR Veterinary Scientific Research Institute (V. I. Gryazin, L. D. Pankratov, K. I. Odarenko, M. Ye. Zhila, and others) developed a method for obtaining associated vaccines against anthrax and emphysematous carbuncles, and anthrax and brucellosis, and the industrial procedures for manufacturing these vaccines were assimilated. Today these vaccines are undergoing testing in a production experiment. Thus associated vaccines will enjoy broad application in veterinary practice in the next few years.

Aerogenic Vaccination

Work is presently being done on the problem of vaccinating animals and birds against various infectious diseases by introducing vaccines in the form of aerosols. Given sensible and precise procedures, these methods should significantly facilitate the labor of veterinarians.

N. I. Aleksandrov, A. Ye. Gefen, et al. (1960) reported development of a method of aerogenic vaccination of sheep against anthrax and against anthrax and brucellosis.

There are also other reports on methods of aerogenic vaccination of animals against anthrax. Thus A. B. Boyakhchyan and L. A. Ambardanyan (1971) and A. B. Boyakhchyan and L. S. Grigoryan (1974) successfully subjected sheep and cattle to aerogenic vaccination against anthrax with STI and GNKI vaccines. Similar results were obtained by A. V. Selivanov and Ye. A. Polyakov (1972), who subjected sheep to experimental aerogenic immunization against anthrax with STI vaccine and against brucellosis with a vaccine prepared from strain 19.

Despite the efficiency of aerogenic vaccination of animals against anthrax by spore vaccines, it may cause some complications. When this vaccination method is employed, large numbers of vaccine spores are liberated in the surrounding environment. As a result the soil becomes densely populated by anthrax microbe spores, even though they are of vaccine origin. It is generally known that veterinarians and kolkhoz and sovkhoz executives are presently concentrating all of their attention on revealing and decontaminating soil foci of anthrax pathogen. Thus the method of aerosol vaccination of animals with live anthrax spore vaccines requires thorough and deep study.

Immunogenesis and Anthrax

Natural infection of animals by anthrax and their recovery from disease are known to cause prolonged and stable immunity. This apparently occurs when disease pathogen of low virulence enters the animal body. This is precisely why an effort has been made to acquire anthrax vaccine exhibiting low residual virulence and possessing high immunogenic properties.

Attempts by a number of researchers to obtain effective killed anthrax vaccines were not graced with success, since the vaccines did not cause formation of stable and prolonged immunity in the animal body.

The following play an important role in development and formation of immunity against anthrax: the genetic features of vaccine capsule-less microbes having the property of immunogenic influence upon the body and the capability for reproducing in the animal body and surviving for long periods of time in the cells of parenchymatous organs; the reactivity and physiological integrity of the animal body, promoting formation of protective substances (antibodies) in response to vaccine microbes.

Recent research attests to the great role played by vaccines in immunogenesis. Data show that vaccine capsule-less microbes, when introduced into the animal body, proliferate and produce a protective antigen (a protective factor), which is what is responsible for the animal's unsusceptibility to anthrax.

That capsule-less anthrax vaccine microbes have a prolonged effect upon the animal body is confirmed by the research of a number of authors.

N. N. Ginsburg (1946) reports that using the bacteriological inoculation method, he was able to isolate STI anthrax vaccine microbes from the place of injection of rabbits and guinea pigs 30 days after the vaccine's administration; subsequently the vaccine microbes disappear (they are eliminated) from the rabbit and guinea pig body.

Studying the time of survival of STI vaccine microbes in the bodies of white mice, guinea pigs, and rabbits, I. A. Chalisov and A. L. Tamarin (1946) detected them in parenchymatous organs by bacteriological inoculation 9 days after the vaccine's injection, and at the place of injection in rabbits 31 days after injection. Performing an experiment on 15 sheep, they isolated STI vaccine microbes from the place of injection after 6 hours and after more than 24 days. The authors point out that this is not the maximum time. Tests on the biological properties of the isolated vaccine microbes showed that they had not experienced any sort of changes. The isolated microbes had biological properties typical of the initial vaccine strain.

Citing the data of the inventor of STI vaccine--Prof N. N. Ginsburg--and his colleagues on the degree of dissemination and elimination* of vaccine microbes, we should note that they do not represent limiting values, since survival of vaccine microbes in the parenchymatous organs of animals is more pronounced and lengthy.

Experiments by S. G. Kolesov, N. A. Mikhaylov, and I. N. Presnov (1962) established extremely lengthy survival of capsule-less microbes of STI and GNKI anthrax vaccines in rabbits and sheep, and their more-pronounced dissemination. The experiments, which were performed in two replications, made use of 92 rabbits and 36 sheep, with 46 rabbits and 14 sheep being immunized subcutaneously with STI vaccine, and 36 rabbits and 22 sheep subjected to subcutaneous immunization with GNKI vaccine. Rabbits were immunized with 2 ml of each of the vaccines at the commonly accepted concentration, while sheep were immunized with one-time injections of 0.25 and 0.5 ml of STI vaccine and 0.3 and 0.6 ml of GNKI vaccine.

Vaccine-immunized rabbits were killed after 10, 15, 20, 30, 50, 75, and 100 days. The degree of microbial dissemination in the rabbit body was studied by means of bacteriological inoculations of large doses (1-2 ml) on BEA in Petri dishes. Inoculant was prepared from blood of the heart, lungs, liver, spleen, kidneys, lymph nodes, brain, and bone marrow, and from material taken from the place of the vaccine's injection. Growth was checked after 1-2 days' incubation in a thermostat.

The research established significant dissemination of vaccine microbes within the rabbit body. STI and GNKI vaccine microbes were isolated from almost all of the organs of the killed rabbits indicated above, both in the first 10-14 days and 30-50 days later. Vaccine microbes were also isolated after 75 and 100 days from individual organs (liver, brain, lymph nodes) of a significant number of killed rabbits. It should be noted that the degree of dissemination in organs was low as a rule, with the exception of individual cases.

Experiments on sheep produced similar results. Survival of STI and GNKI vaccine microbes in parenchymatous organs (liver, spleen, lungs, kidneys, blood from the heart, the place of the vaccine's injection, bone marrow, and brain) was established. Organ cultures were grown by the method described above. Vaccine cultures were isolated from most organs from killed sheep immunized with STI vaccine for a period of up to 75 days, and from killed sheep immunized with GNKI vaccine for a period of up

* Dissemination--penetration of vaccine microbes into organs and tissues of the animal body. Elimination--"disappearance" of microbes from body organs and tissues.

to 100 days. The degree of organ dissemination varied--from 10 to 100 and more colonies in a Petri dish containing BEA.

Microbial cultures isolated from the organs of killed sheep were tested for their cultural and virulent properties. The tests established that in the first subinoculations, the cultures exhibit profuse growth in BEB and produce R- and RO-form colonies on BEA. Later they acquire properties similar to the initial ones. Tests on laboratory animals showed that the isolated microbial cultures have residual virulence typical of the initial vaccine microbes.

Studying the influence of narcotic sleep upon the course of anthrax vaccine infection in white mice, P. V. Bychkin (1958) established significant survival of anthrax vaccine microbes in the animal body. He immunized animals separately with Tsenkovskiy vaccine No 1 and STI vaccine, in doses causing death in only some animals. The white mice that remained alive were subjected to narcotic sleep 20, 30, 45, 50, and 100 days after injection of these vaccines. Urethan was used as the soporific. The anesthetized white mice slept 16-20 hours. After this, the mice woke up feeling normal. However, 2-3 and 5 days after awakening, a significant number of the mice died of anthrax. These experiments also demonstrated prolonged presence of Tsenkovskiy vaccine No 1 and STI vaccine anthrax microbes in the bodies of white mice.

Examining the mechanism behind immunogenesis produced by STI anthrax vaccine, N. N. Ginsburg and his colleagues established by research conducted in 1963 that vaccine spores injected beneath the skin germinated quickly. They could be detected after 3 hours in local lymph nodes, and later in remote lymph nodes, the spleen, the liver, and other organs, where they continue to multiply. Specific anthrax foci form at the place of injection and in areas into which vaccine microbes penetrate; these foci differ from those produced by virulent anthrax microbes in that the most dangerous necrotic component is absent. This specific but qualitatively altered anthrax inflammation can be observed, for example, in immunized sheep for more than 24 days.

N. N. Ginsburg believes that the immunological and immunomorphological mechanism of this inflammation involved formation of protective antigen at the place of proliferation of vaccine anthrax cells. This antigen spreads through the animal's body and causes antigenic stimulation of lymphoid and reticular elements in the lymph nodes and spleen, resulting in their swift transformation into plasma elements. Thus the body undergoes alteration that may be described as formation of antitoxic immunity. Cellular elements of the macrophage type accumulate very quickly both at the place of the vaccine's injection, and wherever the vaccine microbes penetrate. Capturing rods and spores, the microphages digest the former and carry the latter throughout the body. A very complex, as yet incompletely explained process of assimilation, by these elements, of the components produced by the vaccine microbial cells (the antigens) proceeds. These components are transformed into complex compounds (possibly of the antigen- and RNA-macrophage type) which, according to present theory on immunogenesis, perform an informational antigenic function.

N. N. Ginsburg's hypotheses presented here on the mechanism of anthrax immunogenesis are correct from our point of view. They explain the theory of immunity on the basis of new experimental data.

STI vaccine is apparently not the only one having these properties. Other anthrax vaccines containing capsule-less microbes, for example GNKI, Sterne's, Stamatin's, and some other vaccines have them as well. STI and GNKI vaccine microbes remain in the bodies of immunized rabbits and sheep for a longer period of time, up to 75-100 days (the period of our observations). Vaccine microbes probably remain in the body for a longer period of time, though in smaller numbers, and without a doubt they undergo proliferation, synthesizing protective antigen. In response to this, the animal body produces specific protective substances (antibodies). It is precisely by prolonged action of vaccine microbes upon the animal body that we can explain development of very long immunity against anthrax, lasting 1-2 years.

A number of other researchers have also indicated the large role played by the local inflammatory reaction in anthrax immunogenesis.

Studying the role of the postvaccinational reaction in anthrax immunogenesis, F. A. Terent'yev and Ye. P. Stefanova (1946) found the inflammatory reaction accompanying immunization of animals with Tsenkovskiy vaccine No 2 to which saponin is added to be significant. Experiments performed by the authors showed that the time of onset of immunity in response to administration of Tsenkovskiy vaccine No 2 shortens and the antigenic effectiveness of the vaccine rises on the background of an artificially elicited inflammatory process. The authors believe that the significance of the postvaccinational reaction occurring in the development of immunity can apparently be explained by the fact that this reaction creates conditions permitting the antigen to act upon the nervous system, which obviously plays the leading role in immunogenesis in the presence of this disease.

Thus substances added to vaccines as nonspecific stimuli aiding in their dissemination perform the additional role of factors stimulating formation of immunity.

Immunity in Young Animals

Immunity is known to develop differently in young and adult animals. Thus the latter, which have a well developed nervous system and powerful defenses, develop specific immunity in response to injection of immunogenic antigen. Administration of the same antigen to a young animal does not always produce the same effects, which depends on the unique features of immunological reactivity in the early stages of ontogenesis. Reactivity properties at the basis of the body's interaction with infectious pathogens develop gradually in the growing body, assuming final form only at a particular level of the body's general physiological maturation.

A state of reduced reactivity persists in the postnatal period of newborn animals; this is why young animals typically have a weak capacity for producing antibodies in response to active immunization.

These features of immunological reactivity raise the important question of when to vaccinate young animals. The time of vaccination must fall within the period in which the organism acquires the capability for active development of immunity.

Extremely little has been said in the literature about formation of immunity to anthrax in young agricultural animals, particularly in lambs of different age groups. Meanwhile, there is important practical significance to determining the optimum times

to vaccinate lambs against this disease. A series of experiments was performed by G. I. Romanov at the VGNKI [All-Union State Scientific Control Institute of Veterinary Preparations] anthrax and listeriosis preparation control laboratory to answer this question.

The experiments were performed on Tsygay lambs 2, 3, and 4 months old, obtained from ewes that had and had not been vaccinated against anthrax. The animals were vaccinated with STI anthrax vaccine, subcutaneously at a dose of 8 million spores in 0.2 ml. Lambs and sheep were infected with a virulent spore culture of anthrax pathogen, which was injected intracutaneously at a dose of 0.2 ml.

Experiments performed on 249 lambs and 48 sheep revealed that lambs of nursing age are more resistant to anthrax, especially when 2-2.5 months old. As they got older, their susceptibility to anthrax increased. While 70 percent of the 2.5-month lambs obtained from unvaccinated ewes and infected with virulent anthrax pathogen culture (25,000 spores) died, and 80 percent of 3.5-month lambs died, all 4.5-month lambs died. All of the adult sheep also died following injection of 10,000 spores, with most sheep (83.3 percent) dying from a dose of 1,000 spores.

Lambs obtained from vaccinated ewes were more resistant to this disease in all cases than lambs obtained from unvaccinated ewes. The influence of vaccination of the sheep upon the resistance of their offspring manifested itself strongest of all among lambs 2-2.5 months old.

The significant resistance to anthrax displayed by lambs obtained from vaccinated ewes may be explained by presence of specific antibodies introduced with the mother's colostrum and milk, while some degree of resistance to infection in lambs obtained from unvaccinated ewes could be explained by presence of nonspecific immunity factors.

Vaccination experiments performed on lambs 2, 3, and 4 months old showed that the most pronounced immunity is created when animals are vaccinated at an age of 4 months. Lambs immunized at 3 and 4 months maintain strong immunity for not less than 6 months (the period of observation).

A local reaction to administration of the vaccine was absent from lambs of all ages, and among lambs 3.5 and 4.5 months old the local reaction to injection of a virulent culture was characterized by swelling at the place of injection, attaining maximum dimensions of 2×2 cm. A local reaction was absent from lambs infected at an age of 2.5 months.

The temperature reaction of lambs 2, 3, and 4 months old to administration of STI vaccine was more strongly pronounced among the older animals.

Their reaction to vaccine injection proceeds in lambs 2-3 months old as pronounced benign lymphadenitis and splenitis. These processes manifest themselves more intensively in lymph nodes located near the place of the vaccine's injection, and they abate after the 6th day.

The infectious process in lambs of the same age takes the form of a malignant destructive-hemorrhagic process in analyzed organs.

Properties of Anthrax Vaccines

Biological properties: STI and GNKI live spore anthrax vaccines are prepared and dispensed for practical use in the USSR. The vaccines are prepared from capsule-less microbes of standard strains stored in 30 percent glycerin solution or in dry form in a vacuum. The standard anthrax vaccine strains are regularly analyzed for their cultural, reactogenic, and immunogenic properties by the All-Union State Scientific Control Institute of Veterinary Preparations of the USSR Ministry of Agriculture (VGNKI), which sends them to biological plants and combines that make anthrax vaccines from them.

Cultural properties: STI vaccine microbes grown in BEB forma flaky precipitate at the bottom of a test tube, consisting of individual bacilli and long chains; broth above them remains transparent. R-form colonies grow on BEA. GNKI vaccine microbes grown in a test tube containing BEB form a precipitate that readily breaks down in response to agitation; broth above the precipitate contains small flakes consisting of individual rods and short chains. R- and RO-form colonies grow on BEA. STI and GNKI vaccine microbes do not form capsules on coagulated equine serum, and when grown on BEA, they form spores quickly (the former in 4 days and the latter in 4-5 days).

Much attention is devoted to problems associated with keeping standard anthrax vaccine strains stable and testing them, since the quality of these vaccines depends on these factors.

It should be remembered when maintaining standard strains that frequent successive inoculations on nutrient mediums are undesirable (they should be made once every 2 years), since cultural properties may undergo alteration as a result. This is what happened, as an example, with the standard strain for STI vaccine, which was subjected to successive passages on nutrient medium during preparation of several series. As a result it became necessary to isolate a population from a dry standard STI vaccine strain prepared in 1944 by N. N. Ginsburg. This work was done by L. P. Rudenko in 1964-1967. Colony No 1 was isolated as a result. It was used to prepare a standard strain for STI vaccine (colony No 1) possessing more-typical cultural properties in comparison with series 13 standard STI vaccine strain prepared in 1963. While the latter could exhibit diffuse growth in BEB and form RO- and R-form colonies on BEA, the standard strain obtained from colony No 1 clarified the medium as it grew in BEB and formed R-form colonies on BEA.

The cultural and reactogenic properties and the immunogenic activity of the isolated STI vaccine strain (colony No 1) were studied on laboratory and large animals. It was established that it corresponds to the existing requirements in terms of its cultural, reactogenic, and immunogenic properties. Since 1967, the institute has been providing this strain to the biological plants and combines for the manufacture of production series of STI vaccine.

Thus in order to keep standard STI and GNKI anthrax vaccine strains stable, the VGNKI anthrax and listeriosis preparation control laboratory uses initial samples of previously prepared standard strains (in 1964 for STI vaccine and in 1963 for GNKI vaccine), stored in dry form in a vacuum and in 30 percent glycerin solution at 2-4°C, to prepare subsequent series.

Reactogenicity can be defined as the capability of vaccines for causing edema in white mice and guinea pigs at the place of injection of large doses of the biopreparation. This property is typical of vaccines consisting of capsule-less anthrax microbes.

STI vaccine has more-pronounced edema-forming properties than does GNKI vaccine. Even with large vaccine doses the parenchymatous organs of laboratory animals (white mice, guinea pigs, rabbits) do not undergo alteration, owing to which sepsis does not arise in the bodies of the immunized animals either. A certain number of white mice and guinea pigs immunized with STI and GNKI vaccine die as a result of considerable edema at the place of injection of the microbes, and dehydration of the animal's body. Here lies the fundamental difference between these vaccines and the Pasteur and Tsenkovskiy vaccines, the microbes of which are known to have capsulogenic properties.

Immunogenic properties: STI and GNKI anthrax vaccines have strong immunogenic properties.

While Tsenkovskiy's vaccine was tested on laboratory animals (white mice and guinea pigs) only for residual virulence and on rabbits for harmlessness, vaccines produced today by biological enterprises of the Main Administration of the Biological Industry are tested on guinea pigs for immunogenic activity and on rabbits for harmlessness.

As an example the immunogenic activity of GNKI vaccine has been tested on guinea pigs since 1954, while that of STI vaccine has been tested since 1960 by methods developed by S. G. Kolesov, N. A. Mikhaylov, and I. N. Presnov.

The testing of the immunogenic properties of live spore-forming anthrax vaccines on large animals has an extremely long history. L. Pasteur (1881) and L. S. Tsenkovskiy (1883-1887) tested their vaccines for immunogenic activity on large animals: Pasteur used sheep and cattle, and Tsenkovskiy used sheep. The experiments established that sheep are more suited to these purposes, since they are very sensitive to anthrax.

Later (1897-1898) scientists performing comparative commission experiments tried to use, in addition to sheep, cattle and even horses to test the immunogenic properties of Tsenkovskiy's, Pasteur's, and Lange's anthrax vaccines; however, even in these experiments better indicators were obtained with sheep.

Thus the immunogenic properties of Tsenkovskiy's anthrax vaccine and, subsequently, STI and GNKI vaccines were tested only on sheep, and the possibility for correctly evaluating the effectiveness of these biopreparations was established. Important in this case are the doses of the vaccines used in immunization of the animals, the condition of the latter prior to vaccination, and the storage time of the vaccines. Sheep are given dry STI vaccine subcutaneously at a dose of 12 million spores, and dry GNKI vaccine at a dose of 15 million spores. The dose of virulent spore culture of anthrax pathogen used to infect vaccinated and control (unimmunized) sheep is considerable. It is usually equivalent to 10 lethal doses when injected intracutaneously at a volumetric dose of 0.2 ml.

The high immunogenic activity of anthrax vaccines has now been proven.

It should be noted that 1 year may not be the limit of the immunity of animals vaccinated against anthrax, since attempts had not been previously made to test immunity following a longer period of time. It could be hypothesized that animals immunized with STI and GNKI anthrax vaccines maintain immunity for a longer period of time, since apparently its development and extinction are not limited to strictly defined times.

The time of immunity of sheep immunized with STI and GNKI anthrax vaccines was tested for the first time in 1960-1962 2 years after immunization (S. G. Kolesov, N. A. Mikhaylov, I. N. Presnov, Ye. S. Nikolayev, A. G. Mamayev). These sheep were found to be resistant to infection by virulent spore anthrax culture 2 years after vaccination. All vaccinated sheep remained alive, while controls died of anthrax.

A similar experiment was performed on sheep in 1972-1974 by a commission (S. G. Kolesov, L. P. Rudenko, G. I. Romanov, V. I. Solomatin, V. Ya. Yastrebov, V. K. Kretinin, S. M. Timoshenko, V. A. Polyakov, N. A. Bondarenko). Animals immunized in 1972 with STI and GNKI anthrax vaccines at the commonly accepted doses and at doses increased by 1.5 times were infected with a virulent spore culture of anthrax. High immunity was established in tests after 1 year, 1 year 7 months, and 2 years.

The high effectiveness of the STI and GNKI anthrax vaccines used in the country is confirmed not only by acute experiments on sheep testing their immunogenic properties, but also by the results of mass immunization of animals against anthrax. It is known that as a result of regular immunizations of animals against anthrax, epizootics of this disease were eliminated completely from our country more than 25 years ago. In addition to animal vaccinations, the sanitary, diagnostic, and organizational measures implemented by the veterinary service promoted eradication of this disease.

Preparation and Use of Anthrax Vaccines

A culture of vaccine microbes grown on solid agar nutrient medium to the point of consistent spore formation is isolated in 30 percent chemically pure glycerin solution and packed in 50-100 ml flasks (STI vaccine) or subjected to lyophilic freeze-drying in a vacuum in 2-ml vials (STI vaccine and GNKI vaccine). Prior to lyophilization, pasteurized milk is added as the drying medium to concentrated vaccine spore suspension at a ratio of 1:1. The vaccine lyophilization method developed by S. G. Kolesov, N. A. Mikhaylov, and I. N. Presnov has been in use at biological plants since 1960. It insures stability of their immunogenic properties during storage and practical use.

Prepared vaccines are tested for purity of growth by the biological enterprise's biological control section, which cultures them on BEA, BEB, BELB, and Saburo medium. Growth in culture must be typical for vaccine microbes, and it must be pure--that is, free of incidental microflora. After this the vaccines are tested for the concentration of viable spores. This is done by seeding and growing the vaccines on BEA in Petri dishes, after which the resulting colonies are counted. In its working dilution, dry STI vaccine must contain 40 million spores in 1 ml, while dry GNKI vaccine must contain 50 million spores. Live STI vaccine has a concentration within 25-35 million spores per ml.

Every series of vaccines is tested for harmlessness on three rabbits at a dose of 3 ml, injected subcutaneously. The animals must remain alive. The immunogenic activity of anthrax vaccines is tested on guinea pigs. Vaccine from each series is injected twice subcutaneously into 10 guinea pigs--STI vaccine at a dose of 0.1 ml, and at a dose of 0.2 ml 8 days later, and GNKI vaccine at a dose of 0.2 ml, and at a dose of 0.3 ml 8 days later. Twelve days after administration of the second vaccine dose the guinea pigs and 10 control (unimmunized) animals are infected by a multiple lethal dose of Tsenkovskiy vaccine No 2 (250,000-300,000 viable spores). The series of vaccines are said to be immunogenic if they protect a minimum of 8 out of 10 immunized guinea pigs against anthrax, on the condition that a minimum of 8 out of 10 control animals die from anthrax. The method used to test the immunogenic properties of STI and GNKI anthrax vaccines on guinea pigs prior to their release by biological plants and combines for clinical use permits us to evaluate the quality of the biological preparation in immunogenic respects.

L. P. Rudenko (1974) established the possibility for testing the immunogenic properties of STI vaccine on golden hamsters.

The effectiveness of vaccination depends on the state of the animals and the conditions in which they are maintained. Feeding and maintenance, for example, play a significant role in formation of stable immunity in the animal body.

Full-fledged immunity may not always develop when an animal's fatness is insufficient due to unsatisfactory feeding and maintenance. The body reactivity of such animals is low, and the antigenic influence of the vaccine may be inadequate.

Insufficient effectiveness of vaccination of animals exhibiting low body resistance in view of the reasons indicated above may also sometimes lead to other unfavorable results, for example to postvaccinational complications.

The role of body resistance in preventive immunizations with anthrax vaccines, and of arisal of postvaccinational complications, had been indicated by researchers earlier as well (S. N. Vyshelesskiy, 1911-1948; D. L. Nikol'skiy, 1927, etc.). However, at that time attention was usually focused on the biological features of Tsenkovskiy's anthrax vaccine, and it was only occasionally that the influence of other factors upon the outcome of vaccination was noted. Such factors were considered in cases when the causes of complications in vaccinated animals included not only unfavorable weather conditions but also some latent infectious or invasive diseases, ones caused by blood parasites for example.

Much attention was devoted in 1948-1955 to postvaccinational complications in animals immunized against anthrax.

The conducted research (S. G. Kolesov, 1951; P. I. Saley, 1949; P. D. Shat'ko, K. I. Plotnikov, K. P. Voroshilov, D. K. Yermilov, 1951, etc.) established cases of postvaccinational complications in animals immunized with anthrax vaccines owing to activation of latent infections such as pasteurellosis in cattle and sheep, emphysematous carbuncles and babesiosis in cattle, and pathogens producing malignant edema in sheep.

Activation of asymptomatic pasteurellosis infection in rabbits immunized with STI vaccine was experimentally confirmed in experiments conducted by S. G. Kolesov and

A. A. Guttman (1955), and by S. G. Kolesov, N. A. Mikhaylov, B. V. Gorlov, and A. A. Pal'gov (1956). Experiments by E. Sh. Akopyan (1953) confirmed activation of latent emphysematous carbuncle infection in guinea pigs immunized with STI vaccine.

Cases of complications are still encountered among cattle and sheep immunized with anthrax vaccines. Such complications are the result of poor nutrition or unfavorable weather (heat, cold snaps coupled with rain or snow), or crowded maintenance, and so on.

This is why prior to immunizing animals against anthrax, the veterinarian must exclude the presence of the latent infections indicated above, he must not vaccinate animals in intense heat or, on the other hand, in cold, rainy weather, he must not vaccinate animals exhibiting signs of poor nutrition, and he must implement measures to improve their feeding and maintenance.

New Data on the Mechanism of Antianthrax Immunity

Research conducted in the last 20 years changed the old idea about the mechanisms of immunogenesis in the presence of anthrax.

It was established that somatic polysaccharides and capsule glutamic acid polypeptide produced by anthrax bacilli are unable to cause synthesis of antibodies responsible for specific background humoral protection against anthrax. This role is played in anthrax bacillus by a protective antigen--an extracellular proteinaceous substance normally synthesized in the course of the microbe's metabolism in the animal body or on special nutrient mediums, and released into the environment by the bacterial cell.

Sargeant, Stanley, and Smith (1960) demonstrated that protective antigen is the second factor of the three-component synergetic system of extracellular anthrax toxin, discovered in a microbe of this species in 1954 owing to research conducted by Evans and Shocsmith.

Being one of the factors of pathogenicity, the immunogenic component of anthrax bacilli is responsible for formation of antitoxic immunity to this infection (Stanley and Smith, 1963).

Interest in anthrax protective antigen is increasing in different countries with every year. The reason for this lies in the fact that it is a bearer of specific protective properties, and it may be used as an areactogenic, abacillar vaccine.

Moreover research on the protective antigen is revealing important information on the mechanism of anthrax immunity.

Research in this direction has produced fundamentally new facts on the biological basis of the virulence and immunogenicity of anthrax bacilli. The available data attest to the significant role played by anthrax bacillus toxin in the manifestation of many typical traits of infection and formation of specific body defenses. All of this provides the grounds for viewing it as a factor responsible for pathogenesis and immunity in the presence of anthrax.

Vancuris (1970) wrote that the study of anthrax toxin and its components is only the beginning of a major effort, and that only further research could produce a clear picture and additional data on this extremely important biologically active substance.

A typical immunocytic reaction develops in the bodies of animals immunized against anthrax, typified by formation of cells in the lymphocytic and plasmocytic series, which synthesize protective antibodies, and active phagocytes, which possess a set of immunospecific enzymes capable of destroying encapsulated and capsule-less forms of virulent anthrax bacilli, given a sufficiently pronounced macrophagal reaction.

The mechanisms of specific unsusceptibility to anthrax are complex, and they are represented by a system of factors involved in tissue and humoral protection, coupled with more-pronounced differentiation of cellular elements.

Antianthrax immunity is viewed today as anti-infection and antitoxic immunity, and protective antibodies are identified with antitoxins.

Anthrax Toxin

The possibility for generating anthrax toxin in the living organism was indicated long ago by N. F. Gamaleya (1928, 1939). He established that poisonous substances causing the death of rabbits when introduced into their blood are contained in subcutaneous edematous tissue at the place of injection of anthrax bacillus.

The capability of anthrax bacillus for releasing toxin *in vivo* was persuasively demonstrated by Cromartie, Watson, Bloom, and Heckley (1947). They obtained an extract from areas of anthrax edema in rabbits. When injected intracutaneously, these extracts produced the same histological alterations witnessed in response to infection by live bacilli. Moreover the extracts were able to immunize rabbits and, to some extent, guinea pigs and white mice.

A significant step forward in solving the problem of anthrax bacillus toxicity was made by a group of English researchers from (Porton) (Smith and Keppie, 1954; Smith, 1958). Their research demonstrated that the toxin is present not only in edematous fluid but also in blood plasma at a rather high concentration and in thoracic and peritoneal exudate at lower concentrations.

The toxin elicited not only a local inflammatory reaction (edema) coupled with tissue lesion, but also the death of guinea pigs and white mice due to secondary shock. The animals usually died in the first 24 hours, and intravenous injections were found to be especially effective. This toxic substance was first named the lethal factor, but in 1955 the same authors proposed the term "anthrax toxin" and indicated its possible relationship to the protective antigen Gladstone (1946) obtained much earlier *in vitro*.

Anthrax antiserums obtained from horses hyperimmunized by both encapsulated and capsule-less bacillus strains suppressed the edematogenic and lethal action of the toxin, which doubtlessly indicated absence of a direct tie between them and the anthrax capsule. The toxin was also completely neutralized by serum from rabbits immunized with protective antigen.

Evans and Shocsmith (1954) successfully isolated anthrax exotoxin in an *in vitro* experiment. In this case they used filtrates of 7-day cultures grown at 37° in a medium containing casein hydrolysate.

Harris-Smith, Smith, and Keppie (1958) used virulent NPA capsule strain and an atenuated variant of Sterne's capsule-less vaccine to obtain anthrax toxin. The strains were grown at 37° in a mixture of guinea pig serum and trypsin-digested meat, continuously agitated in an atmosphere containing 20 percent CO_2. Both the virulent and the capsule-less vaccine strain synthesized active toxin of identical strength. The toxin exhibited maximum strength 4.5 hours after the start of incubation. Formation and breakdown of the toxin were directly dependent on the quantity of bacteria in the culture. Cultured toxin did not differ in its properties from toxin obtained *in vivo*, it immunized animals, and it was neutralized by equine anti-anthrax serum.

The Components, and Their Immunological Structure

Research by Smith (1958) showed that edematous and lethal factors can be clearly differentiated in native anthrax toxin.

By filtering cultured toxin obtained from anthrax bacillus grown on a medium consisting of acid casein hydrolysate without serum, Thorne, Molnar, and Strange (1960) divided it into two factors. The first passed through a glass filter and possessed the properties of protective antigen, and the second was trapped by the filter, but it was readily eluted with 0.1 M carbonate buffer at pH 9.7. The eluate was named the filter factor. Neither factor was toxic on its own, but their mixture exhibited pronounced toxic action, eliciting inflammatory reactions on the skin of guinea pigs and death of mice.

Study of the filter factor in a gel immunodiffusion reaction using antiserum against "Veybridzh" capsule-less strain demonstrated presence of two precipitation lines differing from the lines produced by protective antigen. It was discovered that the filter factor consists of two nonidentical antigens.

It was established that anthrax toxin synthesized in the body or in culture consists of two factors: an immunogenic antigen (protective antigen) and the filter factor, or the inflammatory component, which is also serologically heterogeneous.

Extending these studies, Stanley and Smith (1961) demonstrated that in addition to anthrax toxin factors I and II, there is also factor III, serologically different from the former two. Factor III was present in toxin formed both by the organism and in culture. On its own it was not toxic, but it amplified the lethal effect on mice when mixed with factor II, but not with factor I. It killed mice and guinea pigs when associated with factors I and II, weakening factor I's capability for eliciting edema in the skin of rabbits and reducing the immunogenic properties of factor II. These authors designated the components of extracellular toxin produced by anthrax bacillus as factors I, II, and III. This nomenclature is used today in many countries.

Beall, Taylor, and Thorne (1962) and others used different names for the toxin: edematogenic factor (EF), protective antigen (PA), and lethal factor (LF), which are correspondingly identical to factors I, II, and III.

Component II is the bearer of protective properties. Addition of component I significantly increases its immunogenicity, while the protective properties of this mixture are reduced by the addition of component III. A mixture of factors I and II

has toxic properties, eliciting an increase in capillary permeability and edema when injected intracutaneously into rabbits and guinea pigs. A mixture of components II and III has lethal properties, causing the death of rats, mice, and guinea pigs. All three components of this toxin (I, II, and III) make up a synergetic mixture simultaneously having edematogenic and lethal action (Lincoln and Fish, 1970). Consequently anthrax bacillus toxin is a three-component system.

The full complex of anthrax toxin synthesized *in vitro* is neutralized by therapeutic antianthrax globulin (Yu. M. Fedotova and A. A. Ulanova, 1970).

Chemical Composition

The chemical nature of each of the three factors of anthrax toxin was studied by Stanley and Smith (1961). According to their data factor I of the toxin contains amino acids, and it is made up of 12 percent nitrogen, 6.4 percent carbohydrates, 0.7 percent phosphorus, and less than 3 percent lipids. The composition of factor II includes 13.9 percent nitrogen, 1.6 percent ash, 3.7 percent glucose, and 0.2 percent phosphorus. Purified factor III preparation was found to contain up to 90 percent protein; analysis reveals 15.1 percent nitrogen, 0.2 percent ash, less than 0.5 percent glucose, and 0.05 percent phosphorus. Paper chromatography demonstrated presence of all amino acids encountered in proteins.

It was subsequently conclusively proved that factors II and III of anthrax toxin are, in molecular respects, heterogeneous proteins, and that factor I is a lipoprotein (Fish and Lincoln, 1967).

According to research conducted by Stanley and Smith (1963), native toxin produced by anthrax bacillus or a mixture of the three purified factors does not exhibit enzymatic activity that could be the basis of the toxic action of this complex.

Pathogenic Action of the Toxin

Much attention was devoted to selecting the laboratory animals to be used in assessment of the activity of anthrax toxin. First all experiments were performed on white mice, guinea pigs, rabbits, and monkeys. They were basically successful.

Beall, Taylor, and Thorne (1962), who studied the pathogenicity of factor III combined with factor II in relation to mice, guinea pigs, and Fisher-344 inbred white rats, established that rats are more sensitive to anthrax pathogen than are mice and guinea pigs.

Toxin synthesized *in vivo* differs from toxin obtained *in vitro* by having faster lethal action and by difficulty of detection (Fish and Lincoln, 1968). All three components of extracellular anthrax toxin are antigenic, and serologically active.

Protective Antigen

Protective antigen owes its discovery to research having the goal of clarifying the nature of the active immunizing element of edematous anthrax fluid.

Bloom, McGhee, et al. (1947), who studied the protective action of edematous anthrax fluid formed at the place of culture injection in rabbits, established that this fluid contains at least two factors: an inflammatory factor (causing tissue damage), and an active immunizing substance. They isolated a fraction that immunized animals from the same fluid, and they called it protective antigen.

Gladstone (1946) was the first researcher to obtain anthrax protective antigen *in vitro*. His experiments (1946, 1948) showed that when anthrax bacillus is grown on a special medium, extracellular antigen that protects healthy animals against experimental infection by anthrax pathogen may be obtained. The immunizing factor was regularly produced by anthrax bacillus in the course of its metabolic activity, and it was released into the nutrient medium.

Gladstone's work refuted the long-reigning idea that immunity may be created against anthrax only with live vaccines or with filtrates of edematous anthrax fluid. This observation was confirmed and further developed in research by M. V. Revo, G. V. Dunayeva, et al. (1960), N. A. Aleksandrova, N. Ye. Gefen, et al. (1961, 1962), Wright, Hedberg, and Slein (1954), and many other authors.

The Mediums, and the Methods of Their Acquisition

Mediums containing native protein of animal origin were used at first to acquire protective antigen.

Gladstone (1946) grew anthrax bacillus cultures in plasma or in animal (sheep, rabbit) serum, and on solid mediums of the same composition at pH 7.12. Washings of cultures grown on solid medium and liquid cultures were centrifuged and filtered through Zeytts plates. When tested on rabbits, sheep, and monkeys, Gladstone's cultural filtrate antigens were found to be just as effective as edematous fluid.

Boor and Tresselt (1955) proposed a new semisynthetic nutrient medium (serial No 243) consisting of serum albumin, yeast extracts, dextrose, salts, and phenol red indicator. The medium was seeded with strain 5-B. Sodium bicarbonate was not used, but carbon dioxide was pumped into flasks containing the medium.

The cultures were incubated at 37° for 16-17 hours, after which they were centrifuged, and the supernatants were passed through a Zeytts filter. The filtrates were stabilized by the addition of 0.1 percent formalin.

Belton and Strange (1954) proposed an original medium containing acid casein hydrolysate.

Other synthetic nutrient mediums were created as well. The first of them was the so-called chemically determined medium 528 (Wright, Hedberg, and Slein, 1954). Its composition included 17 amino acids, adenine, guanine, uracyl, thiamine, bicarbonate of soda, and inorganic salts. Sterile filtrates of anthrax bacillus cultures obtained on medium 528 induced active immunity in guinea pigs, rabbits, and monkeys.

In 1957 Wright and Puziss created a new medium No 1095, which consisted of 15 amino acids, guanine, adenosine, thiamine, pyridoxal, biotin, glucose, sodium bicarbonate, and inorganic salts.

In 1964 Mittal reported on a new semisynthetic medium prepared from casein hydrolysates, bovine serum, and yeast extracts.

In our country, A. V. Mashkov and V. P. Bodisko (1958) were the first to obtain protective antigen *in vitro*. They grew a virulent strain of anthrax bacillus on protein-free medium described by Gladstone and Fields (1940), consisting of casein hydrolysates, to which they added some salts and yeast water.

M. V. Revo, G. V. Dunayev, and V. M. Novikov (1960, 1964) obtained anthrax protective antigen using hydrolyzed nutrient mediums of plant and animal origin, which they inoculated with STI-1 or Shuya-15 capsule-less strains. Later G. V. Dunayev (1968, 1972) improved these mediums and proposed new ones, particularly synthetic protein-free mediums, and he determined the conditions for obtaining anthrax protective antigen with their use.

N. I. Aleksandrov, N. Ye. Gefen, V. F. Runova, and Yu. V. Yezepchuk (1961, 1962, 1963, 1964) proposed a lactate-peptone medium to isolate protective antigen.

Thus there are a large number of nutrient mediums that can be used to obtain anthrax protective antigen. Some of them contain native proteins and are called protein mediums, while others do not contain native proteins and are said to be synthetic mediums.

The Nature of Protective Antigen, and Factors Determining Its Biosynthesis

The chemical nature and physical properties of anthrax protective antigen began to be studied from the first moment of its isolation.

Watson, Cromartie, et al. (1947) performed electrophoretic analysis of native edematous fluid from rabbits experimentally infected with anthrax agents, and they established that protective antigen is a protein occupying, on electrophorograms, an intermediate position between β- and γ-globulins. Protective antigen broke down in response to heating at 57° for 30 minutes, and it underwent digestion by trypsin, which also confirmed its proteinaceous nature. The antigen was found to be stable in a pH range of 5.5-11.0, it did not break down in formalin having a final concentration of up to 1 percent, and it survives for a long period of time in lyophilized form.

The chemical nature of protective antigen synthesized by anthrax bacilli in nutrient mediums was first subjected to study by Strange and Belton (1954). Employing the methods of paper chromatography and analytical ultracentrifugation, they concluded that protective antigen has properties typical of proteins.

Strange and Thorne (1958) studied protective antigen isolated in purified and concentrated form from the filtrate of a culture of Sterne's vaccine strain.

Further study of the chemical properties of purified and concentrated preparations of cultured protective antigen showed that this antigen contains up to 80 percent protein, 3-5 percent nucleic acids, and 10-12 percent polysaccharides.

It was concurrently established that the fraction containing protective antigen had an absorption maximum at 275-280 mm in the ultraviolet spectrum. The antigen

broke down in response to trypsin, as well as heating to 56°, and it was not stable in acid medium (pH 3.0-3.5).

Puziss and Wright (1954) studied the effect of different amino acids on biosynthesis of protective antigen by anthrax bacilli growing in synthetic medium.

The yield of protective antigen depends significantly on the particular producer strain synthesizing the given antigen.

Gladstone's very first observations (1946) demonstrated that protective antigen is synthesized by virulent and vaccine strains of anthrax bacillus exhibiting residual virulence.

Research by G. V. Dunayev (1972) performed on a large number of strains showed that biosynthesis of protective antigen is a hereditary characteristic of anthrax bacillus, and that it is a property of not only vaccine but also virulent strains of this species of microbe.

Studying production of protective antigen in culture, Auerbach and Wright (1955) came to the conclusion that only nonproteolytic strains produce antigen in sufficient quantity.

At the same time Boor (1955) failed to establish a fundamental difference between antigens obtained from the filtrates of cultures of proteolytic and nonproteolytic strains; nor did presence or absence of capsules have significance.

The most active antigen was synthesized in G. V. Dunayev's experiments (1966, 1968) by capsule-less nonproteolytic highly immunogenic STI-1 and Shuya-15 vaccine strains of anthrax bacillus.

Comparison of immunogenic activity and accumulation of bacterial mass showed that microbial cells begin discharging protective antigen into the environment in the exponential growth phase. As the culture ages and cell lysis begins, discharge of antigen into the culture fluid ceases and previously discharged antigen undergoes breakdown.

Concentration and Purification

Various chemical and physical methods have been used to isolate, concentrate, and purify anthrax protective antigen: adsorption, salting out, dialysis, precipitation by acids and alcohol, lyophilic dessication, and gel filtration on Sephadex.

Adsorption of antigen on potash alum was one of the first methods to be used (Wright, Hedberg, and Slein, 1954). This method entailed addition of 0.1 percent alum at pH 5.9 to unpurified antigen.

Wright and Puziss (1957) concentrated protective antigen by lyophilic dessication. But because native material was subjected to drying, purification of the antigen did not occur. The method only made it possible to obtain a larger quantity of antigen per unit volume, and the antigen remained active.

Anthrax protective antigen was also concentrated by salting out with ammonium sulfate (Strange and Thorne, 1958).

Methods involving precipitation of antigen by acids are based on the capability of protein for precipitating out of solution at the isoelectric point. Precipitation of protective antigen by hydrochloric acid was found to be the most effective.

Testing different conditions for concentrating protective antigen with ethanol, N. I. Aleksandrov and his colleagues concluded that antigen precipitates most actively at pH 5.2, and when 40° ethanol is added to the culture fluid.

Protective antigen is isolated today predominantly by sorption on ammonium hydroxide (Yu. V. Yezepchuk, 1968; Puziss and Wright, 1965). The preparation obtained by this method exhibits high immunogenic activity.

Protective antigen maintains its activity for up to 1 year at 4°; according to observations by Puziss and Wright (1963) antigen precipitated by potash alum does not lose its activity for 3 years at 4°.

The Antigen's Immunogenic Activity

Experiments were performed on laboratory and agricultural animals to determine the immunogenicity of protective antigen.

This antigen was tested as an abacillar vaccine in controlled epidemiological experiments, and on people (N. I. Aleksandrov, N. Ye. Gefen, et al., 1963; Puziss and Howard, 1963). In people, it produced weak general and local reactions which disappeared without a trace 2 days after immunization.

Numerous researchers using anthrax protective antigen to vaccinate laboratory animals (white mice, golden hamsters, guinea pigs, rabbits, and monkeys) conclusively proved its high immunizing activity. This capability was highest among preparations sorbed on aluminum hydroxide.

The activity of immunity depended to a significant extent on the activity of the antigen, the dose, the number of injections, and the interval between the preparation's administrations.

According to Ward, McGann, et al. (1965) the number of survivors following control infection among guinea pigs immunized by three doses of protective antigen was higher than among guinea pigs vaccinated by two and one dose.

As far as tests of the biological activity of protective antigen administered on a one-time basis is concerned, this case most clearly reveals the dependence of the immunological response on the model upon which the tests are performed. Almost all guinea pigs immunized with one injection of protective antigen died in response to infection. In relation to rabbits, however, one subcutaneous injection of this antigen affords protection against infection by lethal doses of a highly virulent strain.

As a rule the immunity exhibited by laboratory animals vaccinated with protective antigen is short in duration, not exceeding 150 days.

Auerbach and Wright (1955) observed an interesting phenomenon: Protective antigen exhibited protective action in experiments on guinea pigs only when the latter were infected by certain virulent strains of anthrax bacillus. This did not occur with rabbits. Later the authors demonstrated that protective antigen produced by "deviant" strains protected pigs only from homologous infection, and it would not afford protection against heterologous infection.

No less interesting in this regard is research by Personeus, Cooper, and Persival (1956), who reported that the more-virulent cultures of anthrax bacillus are less capable of surmounting acquired immunity than is a suspension of reduced virulence having a higher concentration of cells.

We (G. V. Dunayev) discovered back in 1961 that combined immunization by protective antigen and live spore vaccine significantly raises the resistance of animals to infection by virulent anthrax cultures.

The same was reported independently of our research in 1962 by the American scientists Klein, De Armon, and Lincoln. They determined mathematically that combined immunization with protective antigen followed by live vaccine increases an animal's resistance by more than 100 million times in comparison with controls.

We subsequently performed experiments on 60 guinea pigs to determine the effectiveness of combined vaccination. The same scheme of combined vaccination was applied to all animals: First we injected live STI vaccine, and 10 days later we performed a triple immunization with protective antigen. Combined vaccination promoted the most effective protection against anthrax, even despite the fact that a significant dose of Tsenkovskiy vaccine No 2 spores (100 million) was used for control infection. All 60 guinea pigs vaccinated by the combined method survived, while all control animals died.

Within the first years of its acquisition, protective antigen was tested as an abacillar vaccine on agricultural animals.

Gladstone (1946) immunized three sheep with protective antigen and established their resulting immunity to anthrax.

Boor and Tresselt (1955) tested ethanol-isolated protective antigen on sheep; 120 mg of such antigen, injected subcutaneously three times at 6-day intervals (20, 40, and 60 mg) protected all six immunized sheep against infection by 120,000 spores of a virulent anthrax strain 84 days after immunization.

Performing acute experiments involving infection of cattle, sheep, and pigs, Schlingman, Devlin, Wright, et al. (1956) basically confirmed the immunizing activity of anthrax protective antigen in relation to agricultural animals, and simultaneously demonstrated the need for repeat immunization to insure full immunity.

Jackson, Wright, and Armstrong (1957) studied the immunogenicity of alum antigen, prepared on synthetic medium 599, in experiments on cattle. Vaccination of the animals resulted in pronounced immunity to anthrax. Resistance was effective 1 month following two-time immunization. After 3.5 months the activity of immunity declined, but it was detected in some animals after 7 months. One-time vaccination created relatively unstable immunity. In comparison with protective antigen, live spore vaccine exhibited better results.

N. I. Aleksandrov, N. Ye. Gefen, et al. (1961) vaccinated sheep with protective antigen precipitated by alum from cultural filtrates obtained from an STI-1 strain grown on lactate-peptone medium.

The preparation was injected twice with an interval of 30 days, and the total antigen dose was 600 mg. Out of 15 immunized sheep infected by 100 DCl spores of virulent strain No 836 20 days after the second vaccine administration, 14 survived.

Tests of protective antigen were performed on agricultural animals by domestic and foreign authors in order to create new abacillar immunizing preparations which, in contrast to live anthrax vaccines, would be areactogenic and safe, but at the same time possess good protective properties.

It has now become obvious that as an abacillar vaccine used to immunize agricultural animals against anthrax, protective antigen has no advantages over modern live spore vaccines. This preparation would better be used to immunize people, as required by the medical indications.

Further study of combined immunization of animals by live spore vaccine and protective antigen offers special interest.

Humoral Protective Factors and the Immunomorphological Aspects of the Body's Postvaccinational Reorganization

The nonspecific factors of the body's humoral protection against anthrax were first subjected to study as long ago as at the end of the last century.

V. V. Groman reported in 1884 that fresh animal blood has bacteriostatic action in relation to anthrax bacillus. Groman's observations were confirmed by Fodor's experiments (1887). Some months later Buchner (1889) established that serum from animals of some species is bactericidal in relation to anthrax bacillus.

According to Petterson (1936) normal serums have bactericidal action in relation to anthrax microbes because of presence of special substances in serum--β-lysins. Bloom, Watson, et al. (1947) also isolated these substances from the tissues of various animals, giving them the general name "anthracocides".

The bactericidal activity of serum from animals of different species varies. It manifests itself most clearly in serum from rabbits, horses, and dwarf pigs, the bactericidal activity of serum from guinea pigs is extremely weak, and it is absent from the serum of mice, sheep, cattle, the common breeds of pigs, dogs, and chickens (I. V. Sokolova, 1955; Staub, 1949; Kimura, 1967).

Attempts at establishing a correlation between the bactericidal activity of normal serums from highly sensitive animals and that of serum from animals naturally resistant to anthrax were unsuccessful (V. Kh. V"lchanov, 1966). Nor are there any grounds for associating disintegration of bacilli with the bactericidal properties of immune serums. Research by Petterson (1928) and Sobernheim (1929) showed that the bactericidal activity of serums from animals immunized against anthrax differs a little from that of normal serums taken from the same animals.

Lysozyme is also a humoral factor of nonspecific anthrax immunity. In addition to having bactericidal functions in relation to anthrax microbes, lysozyme has opsonic action, and it may promote more-intense phagocytosis.

Anthrax immunity is typified by lack of differentiation in specific immunological reactions, and by difficulties in revealing protective antibodies.

It is commonly known that when anthrax bacilli are repeatedly injected into animals (first vaccine and then virulent strains), highly active therapeutic serum saturated with antibodies can be obtained.

It is practically impossible to detect antibodies (as commonly defined) in therapeutic antianthrax serum that passivelyprotects animals immunized with it. This is why therapeutic antianthrax serum is titrated in biological experiments on laboratory animals.

As of today, protective antibodies effective against protective antigen have been discovered by the complement-fixation reaction, the RDP [not further identified], and by the (Kuns) indirect immunofluorescence method in serums from animals vaccinated with anthrax protective antigen and live spore vaccines (G. V. Dunayev, 1972; Thorne and Belton, 1957; McGann, Stearman, Wright, 1961, etc.).

It was established by the immunofluorescence method that when guinea pigs are vaccinated with protective antigen, antibody-containing cells (plasma cells) accumulate in lymphoid organs. In the early stages of immunogenesis, antibodies are detected in hemocytoblasts and in juvenile plasma cells, while at the peak of the immune response mature plasma cells are the principal antibody-containing elements. Presence of antibodies has also been noted in small lymphocytes. Antibodies complementary to protective antigen are clearly detected by the luminescent-serological method in immune serums from animals at times when they display high resistance to infection by lethal anthrax culture doses.

Anthrax immunity is basically interpreted as antitoxic immunity, and protective antibodies are identified with antitoxins (N. N. Ginsburg, 1966; Ward, McSann, et al., 1965).

The morphology of immunogenesis brought on by anthrax vaccination was studied by I. A. Chalisov and A. L. Tamarin (1946), N. Ye. Gefen and G. Ya. Gordon (1961), B. S. Gusman and T. V. Migulina (1966), E. N. Shlyakhov, I. G. Shroyt, and T. A. Burdenko (1967), and R. Slavchev, D. Stoyanov, et al. (1967). Experiments were performed on guinea pigs, rabbits, and sheep given different quantities of live spores produced by vaccine strains: STI-1, $34F_2$, Ikhtiman, and 1190-R.

The results of these studies may be summarized as follows. One day after inoculation by the studied strains, inspection of regional lymph nodes revealed hyperplasia of germ centers in secondary nodules, some dilation of sinuses, endothelial swelling, and proliferation of reticular cells, many of which exhibit differences in mitotic pattern. The changes increase in proportions by the 14th day, and they are typified by appearance of a large number of plasma cells. Then the changes gradually attenuate, but a significant number of plasma cells can still be detected on the 60th day. Remote lymph nodes, the spleen, bone marrow, and lymphoid elements in the lungs become involved in the vaccine process. An intensive shift in cell composition toward plasma cells occurs in these organs.

When animals are immunized with live spore vaccines, the plasmocytotic reaction becomes widespread: Not only regional lymph nodes but also lymphoid accumulations far away from the place of application of the vaccine become involved. The immunogenesis process takes up to 5 months in this case, and immunity is high.

Phagocytosis

I. I. Mechnikov (1903) showed that phagocytosis plays a prominent role in natural immunity to anthrax. According to Mechnikov the mechanism of this immunity consists exclusively of phagocytosis: Anthrax bacilli are captured by leukocytes, and they perish within them.

He established that the leukocytes of frogs, chickens, and pigeons, which are naturally unsusceptible to anthrax, intensively phagocytose anthrax microbes.

On the other hand extremely few phagocytes were capable of engulfing anthrax bacilli in rabbits and guinea pigs--animals that are especially susceptible to anthrax infection. The picture was entirely different with vaccinated animals (rabbits, rats): Their leukocytes accumulated in large numbers at the place of injection of the infecting material, and they captured and destroyed bacilli. Active phagocytosis was also established in rabbits given antianthrax serum.

These facts gave Mechnikov the grounds for interpreting phagocytosis as one of the principal mechanisms of antimicrobial immunity against anthrax.

It became clear that phagocytosis has great significance to surmounting anthrax infection or to anthrax immunity. In addition to this, the exceptional role played by specific immune serum, as a factor intensifying the activity of phagocytes, was demonstrated. This function of immune anthrax serum was explained by presence of opsonins within it (Cromartie, Bloom, and Watson, 1947).

Normal serums do not possess this property. Thus the bactericidal activity of serums from chickens and pigs in relation to anthrax bacillus is practically zero, while phagocytosis in the nonimmune organism is high. High bactericidal activity of serum and low phagocytosis are noted in rabbits and horses (A. L. Toshkov, A. S. Toshkov, et al., 1964).

Research on the phagocytic reaction to anthrax infection focused significant attention on determining the role of the anthrax bacillus capsule in this process (Smith and Gallop, 1956). The research showed that capsules protect anthrax bacilli against phagocytosis; however, the mechanism of their protective action has not been fully clarified.

Bruckner, Kovacs, and Denes (1953) explain the antiphagocytic activity of capsule glutamine polypeptide by the significant negative charge of this substance.

In turn, Keppie, Harris-Smith, and Smith (1963) reported that anthrax glutamine polypeptide does not have a direct injurious effect on phagocytes, and that its action manifests itself as suppression of opsonization of microbial cells by serum antibodies.

Studying the mutual relationships between anthrax bacilli and phagocytes, N. N. Ginsburg and Yu. M. Fedotova (1963) performed experiments with cultures of human embryonic cells, and they came to the conclusion that capsules protect bacilli not only against phagocytosis but also against bactericidal substances produced by tissues, as has been believed previously. Owing to capsules, virulent anthrax bacilli are capable of attaching two cells, and thus coming in close contact with the latter, actively influencing them with their toxin.

Considering that active mesenchymal elements (macrophages) play the decisive role in immunity, N. N. Ginsburg and T. N. Maslova (1963), T. N. Maslova (1965), and Yu. M. Fedotova (1968) performed a series of experiments to study the capability of macrophages from aseptic exudates in the abdominal cavity and spleen of immune and nonimmune guinea pigs for phagocytosing anthrax bacilli. They revealed a mutual influence between macrophages and encapsulated bacilli during phagocytosis of a virulent strain. On one hand macrophages phagocytosing encapsulated chains were subjected to degenerative alterations, while on the other hand individual cells in encapsulated chains that were only partially engulfed also underwent disintegration. The authors explained this phenomenon as remote action of macrophages upon bacilli situated outside cells. Complete phagocytosis often occurred when macrophages phagocytosed virulent forms of anthrax bacillus.

Protective antigen--a component of bacillar toxin responsible for immunological reorganization of the body--has a clearly expressed capability for stimulating phagocytosis. According to observations by G. V. Dunayev (1972), intensive phagocytosis of a capsule strain of anthrax bacilli by neutrophilic blood leukocytes is noted in guinea pigs immunized with protective antigen (Figure 25). The high phagocytic activity of neutrophils correlates with the resistance of animals to infection by lethal doses of anthrax culture. An intense macrophagal reaction, characterized by a complete immunological response, develops at the focus of antigenic stimulation in vaccinated animals. A significant number of macrophages become actively included in phagocytosis, eliciting destruction of absorbed bacilli (Figure 26).

Thus phagocytosis plays a significant role in the body's defense mechanism against anthrax infection, and it is one of the factors predetermining its outcome.

Allergy

Allergy develops in an animal that is vaccinated, or one that survives a case of anthrax.

Zironi (1924) was the first to report the possibility of reproducing a skin allergy phenomenon in animals sensitized by injection of live or formalin-killed anthrax bacillus cultures.

Allergic reactions taking the form of local inflammatory processes were also observed by Hruska (1927) in horses subjected to repeat subcutaneous injection of anthrax-infected edematous fluid.

Experiments on guinea pigs by Combiesco (1929) established that microbe-free autolysates of anthrax bacillus cultures and dissolved somatic polysaccharides do not have sensitizing action. On the other hand injection of weakened cultures

Figure 25. Phagocytosing Neutrophilic Leukocytes: Luminescent Microscopy. ML-2, MNF-10. Objective 90 × 1.25; Ocular Photo 012.5x.

Figure 26. Phagocytosing Macrophage. Destruction of Captured Bacillus: Phase-Contrast Microscopy. ML-2, KF-4, MNF-10. Objective 90 × 1.25; Ocular photo 012.5x.

into guinea pigs is accompanied by development of heightened sensitivity to anthrax bacillus antigens.

Veterinarians A. Petrov and Ye. Kiselev (1937) proposed the allergen antraksin and developed a method of allergic diagnosis of anthrax in animals. The proposed preparation was tested on rabbits, guinea pigs, pigeons, and cattle. Intracutaneous injection of antraksin into animals infected by anthrax pathogen or vaccine caused development of a sufficiently well pronounced inflammatory reaction in just 6-12 hours, typified by specificity and possessing diagnostic value.

The first domestic preparation--anthrax allergen of the Moldavian SSR Scientific Research Institute of Epidemiology, Microbiology, and Hygiene--was created in 1957 (E. N. Shlyakhov, 1958). This is sterile edematous fluid, preserved by the addition of 0.05 percent formalin, from guinea pigs infected subcutaneously with STI-1 vaccine strain.

Later this allergen was improved somewhat and released with the name "antraksin" (E. N. Shlyakhov, 1960).

Tests of the allergen by the author on guinea pigs, rabbits, and sheep immunized with STI vaccine demonstrated its sensitivity and specificity. In 1961 E. N. Shlyakhov and S. A. Shvarts developed the procedure for obtaining chemical antraksin, which they isolated directly from microbial mass produced by STI-1 vaccine strain by means of mild hydrolysis following preliminary thermal denaturation of ballast proteins.

Antraksin is a pink transparent liquid with a pH of 7.2-7.4. This preparation was not inferior to previously obtained allergens in terms of its sensitivity, and it had a number of advantages: It was practically devoid of sensitizing properties associated with native animal proteins, and it did not have immunizing action. According to E. N. Shlyakhov and S. A. Shvarts (1964), biologically complete chemical antraksin consists of a protein-polysaccharide-nucleic complex.

The opposite opinion exists as well. It is believed that anthrax bacillus fractions consisting predominantly of protein have allergic activity (V. F. Runova and O. A. Rudneva, 1968).

BIBLIOGRAPHY

1. Aleksandrov, N. I., Gefen, N. Ye., Garin, N. S., and Gapochko, K. G., ZHMEI, No 10, 1960, p 34.

2. Boyakhchyan, A. B., and Grigoryan, S. L., "Voprosy effektivnosti protivosibireyazvennykh meropriyatiy" [Questions of the Effectiveness of Anthrax Control Measures], Moscow, 1974, p 102.

3. Bezredka, A. M., "Ocherki po immunitetu" [Notes on Immunity], Moscow-Leningrad, 1929.

4. Burgasov, P. N., Cherkasskiy, B. L., Marchuk, L. M., Shcherbak, Yu. F., "Sibirskaya yazva" [Anthrax], Moscow, 1970.

5. Vyshelesskiy, S. N., "Vaktsiny sibirskoy yazvy i protivosibireyazvennaya syvorotka, ikh polucheniye i primeneniye na praktike" [Anthrax Vaccines and Antianthrax Serum, Their Acquisition and Practical Use], St. Petersburg, 1911.

6. Ginsburg, N. N., "Rukovodstvo po mikrobiologii, klinike i epidemiologii infektsionnykh bolezney" [Handbook of the Microbiology, Clinical Pattern, and Epidemiology of Infectious Diseases], Moscow, 1966, p 295.

7. Ginsburg, N. N., and Maslova, T. N., ZHMEI, No 4, 1963, p 62.

8. Gryazin, V. I., Odarenko, K. I., Mishutin, A. F., Zuyev, V. G., and Zhila, M. Ye., "Assotsiirovannaya vaktsinatsiya sel'skokhozyaystvennykh zhivotnykh" [Associated Vaccination of Agricultural Animals], Alma-Ata, 1972.

9. Dunayev, G. V., "Tezisy dokladov 9-go Mezhdunarodnogo kongressa po mikrobiologii" [Abstract of Reports at the Ninth International Congress on Microbiology], Moscow, 1966, p 653.

10. Dunayev, G. V., "Bor'ba s boleznyami sel'skokhozyaystvennykh zhivotnykh. Nauchnyye trudy Khar'kovskogo Zoovet. instituta" [Control of Diseases of Agricultural Animals. Scientific Works of the Khar'kov Zooveterinary Institute], Khar'kov, Vol 3, 1968, p 9.

11. Kolesov, S. G., and Borisovich, Yu. F., BIOPREPARATY, VIRUSY, MIKROBY, Moscow, No 7, 1955, p 5.

12. Kolesov, S. G., and Mikhaylov, N. A., BIOPREPARATY, VIRUSY, MIKROBY, Moscow, No 6, 1966, p 242.

13. Kolesov, S. G., Mikhaylov, N. A., and Presnov, I. N., TRUDY GNKI, Moscow, Vol 11, 1962, p 124.

14. Kolyakov, Ya. Ye., "Veterinarnaya mikrobiologiya" [Veterinary Microbiology], Moscow, 1960, p 20.

15. Levina, Ye. N., and Kats, L. N. ZHMEI, No 10, 1964, p 85.

16. Mechnikov, I. I., "Nevospriimchivost' v infektsionnykh boleznyakh" [Unsusceptibility to Infectious Diseases], 1903.

17. Nikol'skiy, D. L., VESTNIK SOVREMENNOY VETERINARII, No 13, 1927, p 388.

18. Revo, M. V., Dunayev, G. V., and Novikov, V. M., VETERINARIYA, Kiev, No 2, 1964, p 47.

19. Rozen'yer, L. A., "Sibirskaya yazva u cheloveka" [Anthrax in Man], Kishinev, 1948.

20. Romanov, G. I., TRUDY GNKI, No 17, 1971, p 235.

21. Rudenko, L. P., TRUDY GNKI, No 14, 1967, p 234.

22. Selivanov, A. V., and Marinkova, V. V., "Voprosy effektivnosti protivosibireyazvennykh meropriyatiy," Moscow 1974, p 103.

23. Terent'yev, F. A., SOVETSKAYA VETERINARIYA, No 11, 1934, p 9.

24. Terent'yev, F. A., and Stefanova, Ye. P., VETERINARIYA, No 10, 1946, p 11.

25. Tsenkovskiy, L. S., AVN, Book 4, 1884, p 108.

26. Tsenkovskiy, L. S., "Sb. rabot Khersonskogo zemstva" [Collection of Works of the Kherson Zemstvo], Vol 3, 1886.

27. Shalashnikov, A. P., "Sb. trudov Khar'kovskogo veterinarnogo instituta" [Collection of Works of the Khar'kov Veterinary Institute], Vol 2, 1888, p 361.

28. Shat'ko, P. D., Plotnikov, K. I., Voroshilov, K. P., and Yermilov, L. K., VETERINARIYA, No 5, 1951, p 34.

29. Chalisov, I. A., and Tamarin, A. L., SBORNIK RABOT NIIEG, No 1, 1946, p 102.

30. Shlyakhov, E. N., "Antraks" [Anthrax], Kishinev, 1964, p 29.

31. Auerbach, S., and Wright, G. G., J. IMMUNOL., Vol 75, No 2, 1955, p 129.

32. Belton, F. C., and Strange, R. E., BRIT. J. EXPTL. PATHOL., Vol 35, No 2, 1954, p 144.

33. Cromartie, W. J., Bloom, W. G., and Watson, D. W., J. INFECT. DIS., Vol 80, No 1, 1947, p 1.

34. Ivanovics, G., and Foldes, J., ACTA MICROB. HUNG., Vol 5, No 1, 1958, p 89.

35. Jackson, F. G., Wright, G. G., and Armstrong, J., AMER. J. VET. RES., Vol 18, No 69, 1957, p 771.

36. Keppie, J., Harris-Smith, P. W., and Smith, H., BRIT. J. EXPTL. PATHOL., Vol 44, No 4, 1963, p 446.

37. Lincoln, R. E., and Fish, D. C., "Anthrax Toxin. Microbiol. Toxins," New York, Vol III, 1970, p 361.

38. Sargeant, K., Stenley, J. L., and Smith, H. J., GEN. MICROBIOL., Vol 22, No 1, 1960, p 219.

39. Smith, H., and Keppie, J., NATURE, No 173, 1954, p 869.

40. Stanley, J. L., and Smith, H. J., GEN. MICROBIOL., Vol 31, No 2, 1963, p 329.

41. Strange, R. E., and Belton, F. C., BRIT. J. EXPTL. PATHOL., Vol 35, No 2, 1954, p 153.

42. Strange, R. E., and Thorne, C. B., J. BACTERIOL., Vol 76, No 2, 1958, p 192.

43. Vancurik, J., CESKOSL. EPIDEM. MICROBIOL., IMMUNOL., Vol 19, No 3, 1970, p 118.

ANTHRAX IN PIGS

Historical Information

The question as to the susceptibility of pigs to anthrax remained debatable for a long time. Some scientists thought that pigs were resistant to this disease in natural conditions, and that they do not fall ill in response to artificial infection. This opinion became especially entrenched after Brauell (1888), Kitt, Nocar, and Klein (1890), and others were unable to experimentally elicit illness in pigs. It was not until 1894 that A. K. Chernogorov and V. Ye. Vorontsov were able to reproduce the disease in pigs with a lethal outcome, thus proving that these animals are susceptible to anthrax.

Occurrence

Well and Weisse observed anthrax in pigs at two places in Morocco in 1925-1926. Almost all stricken animals died. An anthrax outbreak is known to have occurred in Indonesia, where almost 50 percent of the animals died during one epizootic (Eikmann, 1930).

Stein (1953) writes that in a period of 30 years, anthrax outbreaks were observed among pigs in 43 states. In 1952 195 pig carcasses infected by the anginal form of anthrax were condemned at slaughterhouses of the USA. According to data available for the USA, the number of cases of anthrax recorded among pigs totaled 1,088 in 1951, 1,614 in 1952, 127 in 1953, and 123 in 1954. According to Goldstein (1957) 100 tons of meat and bone meal were imported into the state of Ohio in 1952; this meal was mixed with concentrates and sold to farmers. Anthrax broke out at 258 farms from 22 February to 26 October 1952. Three hundred eight-four pigs died.

Cases of pigs catching anthrax were also observed at the Moscow meat-packing plant. In 10 years (1959-1968), veterinary expert examination of carcasses revealed 71 cases of anthrax localized in submaxillary lymph nodes, and only in one case was the septic form noted.

During this same time 242 pigs with anthrax localized in submaxillary lymph nodes were revealed in the country by inspections performed prior to slaughter. Expert examinations performed after slaughter revealed anthrax pathogen in 887 pig carcasses. This demonstrates a need for continuing research on the disease and improving veterinary expert examination of carcasses, especially with the purpose of diagnosing different forms of anthrax in pigs.

The Pathogen

The pathogen isolated from the bodies of pigs suffering chronic disease has certain unique features, and it can sometimes be distinguished by its cultural-morphological and virulence properties.

Microscopic analysis of smears prepared from submaxillary, retropharyngeal, cervical, and mesenteric lymph nodes as well as lesions in the spleen, kidneys, liver, and lungs as a rule reveals morphologically altered forms of anthrax microbes.

Studying the cultural-morphological and virulent properties of 176 strains isolated from sick pigs, we concluded that the degree of morphological alterations experienced by bacilli depended on the time the pathogen was present in the animal body. When BEA is inoculated in the first phase of disease, microbes grow as R-form colonies; then they dissociate into one or several type S variants, and the intermediate O-form. Pathogen isolated from a lesion focus noticeably differs from the initial type in its morphology, nature of growth on nutrient mediums, and degree of virulence. Morphologically altered *Bac. anthracis* form smooth colonies without curls. Colonies exhibiting insignificant patterns or ones having the appearance of a loose spider-web--erosive forms (Figure 27)--are sometimes encountered; dwarf colonies are also seen. They assume the consistency of slime when grown on blood and sloped agar. Such strains grow diffusely in broth to form a ring around the wall of the culture vessel in 3-4 days; an amorphous residue forms at the bottom of a test tube. The microbes described here differ morphologically from typical forms in that rather than long chains or strands, they form thickened, shorter, randomly oriented rods in culture. Such strains cause insignificant hemolysis on blood agar. Sometimes anthrax microbes obtained from lymph nodes were not pathogenic in relation to white mice and 15-20-day guinea pigs, while the precipitation reaction with antigen from such microbes was positive. However, they did become pathogenic following passages on nutrient medium, not only to white mice but also to adult guinea pigs.

Weakly virulent strains preserve the capability for spore formation, and their antigenic (as determined by the precipitation reaction) and biochemical properties. Such strains of anthrax pathogen caused the death of white mice in 10-20 days and more.

Far from all strains isolated from pigs underwent dissociation from the R- to the S-form. Some strains that had been present for a long period of time in the animal body exhibited pronounced dissociation when grown on nutrient mediums. When subjected to repeat inoculations, some of them constantly produced the typical R-form, and then within a very short period of time they ceased their growth and died. Others, on the other hand, produced different variants in just a few days after inoculation, in which case the morphological alterations experienced by the colonies did not always correspond to biological alterations. Out of 176 strains that we tested, four produced the typical R-form in control cultures, they acquired motility, and their spores were oval in shape; in this case virulence was significantly lower than that of some bacilli from S-form colonies.

Epizootiology

Pigs get anthrax irrespective of age, sex, and breed. However, animals more than 4 months old are the most susceptible. Disease may arise at any time of the year,

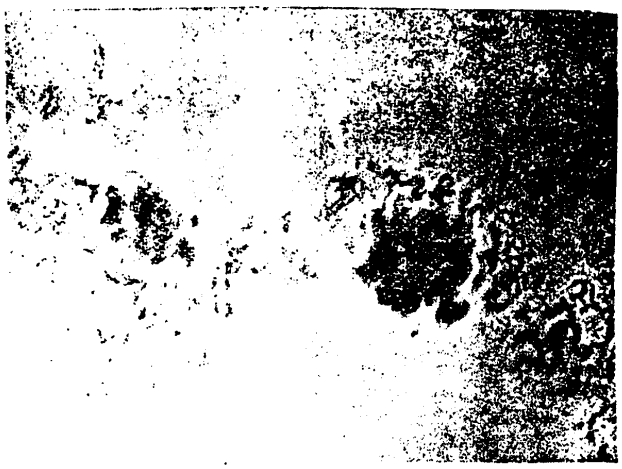

Figure 27. Erosive Colonies

but it usually appears in summer, due to presence of infection transmission factors. The latter can include feed and water contaminated by the discharges of sick animals, and the carcasses of rodents and other animals killed by anthrax. However, the soil is believed to be the principal reservoir of anthrax pathogen.

Animal burial pits and places of interment of anthrax-infected carcasses are stable foci of the causative agent. The author observed cases of arisal of anthrax among pigs that had dug up burial pits 53 years old. The territory of former tanneries and places where animals killed by anthrax had been autopsied and veterinary and sanitary rules had not been complied with may be foci of infectious pathogen.

In some areas anthrax arises sporadically, while in others infections are recorded over long periods of time (points of permanent infection).

Pigs are sometimes infected when fed the meat of animals killed by anthrax.

Stray pigs chancing upon animal burial pits may infect themselves by anthrax similarly. They may also infect themselves by eating feces and the carcasses of dead rats carrying anthrax pathogen.

Sick pigs may carry bacilli for a long period of time, and in this case they become responsible for the spread of anthrax.

Pigs are infected mainly by the alimentary pathway. Penetration of the pathogen through skin is rarely observed, and it is only possible when the latter's integrity is disturbed (by scratches, abrasions, and wounds) or due to bites by biting insects.

Pathogenesis

Anthrax usually proceeds in pigs in the form of a local pathological process, and much more rarely as septicemia.

Insignificant injuries of the mucous membrane covering the oral cavity and the digestive tract promote penetration of the pathogen into local lymph nodes, and then into the bloodstream. However, the microbe does not proliferate in blood during the first stage of disease due to the latter's bactericidal properties. Later on, microbes reproducing in blood liberate toxins that paralyze the phagocytic activity of leukocytes and cells of the reticuloendothelial system, which promotes unhindered proliferation of anthrax pathogen. As a result general septicemia develops in short time, and the animal dies.

Arisal of edematous swellings typical of anthrax may be explained by accumulation of large quantities of capsule substance in places of intense proliferation of the microbes, which prevent circulation of tissue fluid in some organs and areas of the body, while arisal of hemorrhages in the spleen, liver, brain, and lymph nodes can be explained by injury to capillaries by masses of bacilli accumulated within them; this also promotes formation of carbuncles and edematous regions in internal organs. The inability of blood to coagulate and its varnish consistency in killed animals can be explained by the pathogen's capability for producing hemolysin.

Anthrax develops locally (in its anginal form) as a result of penetration of the infectious agent through lesions in the mucosa of the oral cavity. Insignificant injuries to the mucous membrane of the gastrointestinal tract also promote the animal's infection.

In pigs, submaxillary and retropharyngeal lymph nodes are afflicted more frequently (in 95 percent of the cases) than are other nodes and parenchymatous organs.

It is believed that an animal can fall ill following administration of anthrax microbes in different ways: subcutaneously, intravenously, intraperitoneally, and through the respiratory tract. Disturbance of the integrity of mucous membranes of the oral cavity, the gastrointestinal tract, and the respiratory organs is a mandatory prerequisite of infection.

Anthrax may proceed wtih predominant affliction of the intestine and lungs. D. M. Teternik, A. V. Katagoshchin, N. M. Bogdanov, L. S. Nevskiy, Elsasser (1919), Siebel (1914), Glage (1914), Schmidt (1913), and Schlegel (1913) interpret the intestinal form of anthrax in pigs not as an independent but rather as a secondary infection developing by the hematogenic pathway. V. Yu. Vol'ferts (1924) Passerini (1907), Freese (1909), and Dommann (1910) hold the same opinion relative to pathogenesis of the pulmonary form, interpreting it to be a result of hematogenic infection. But arisal of the pulmonary form of anthrax is associated with mandatory primary sensitization of the epithelium of the respiratory tract by inhaled spores of anthrax microbe. Subsequent penetration of anthrax pathogen into the mucous membrane of the epithelium leads to development of the pathological process in the lungs.

We performed special experiments on 14 animals 8-12 months old weighing from 60 to 100 kg in order to study the pathogenesis of the anginal form of anthrax. The animals were infected by strains isolated from a pig that had suffered the anginal form of anthrax. The obtained culture was washed from agar and encased in gelatin capsules, 10 of which were administered *per os* to 3 experimental animals. The oral mucosa of three other pigs was severely injured prior to infection, while that of four others was subjected to mild injury. Then the animals were fed feed containing anthrax microbe spores, or material containing spores was rubbed into a wound.

A day-old broth culture was injected into the submaxillary region of two pigs with a syringe at a dose of 0.5 ml. One pig was fed an infected lymph node, and spores were rubbed into uninjured oral mucosa of the other pig.

Experiments showed that only those pigs with a severely damaged oral mucosa receiving pathogen culture *per os* and pigs infected subcutaneously in the submaxillary region contracted anthrax. Animals with normal and mildly injured mucosa could not be infected even when spores were rubbed in with a brush. This is confirmed by experiments performed by A. K. Chernogorov, V. Ye. Vorontsov, D. M. Teternik, and other researchers, and by practical observations.

A. V. Katagoshchin suggests the opinion that *Bac. anthracis* readily penetrates through the mucous membranes of the tonsils, and he even interprets them as a unique sort of wound surface. He also believes that affliction of the tonsils only affects lymph nodes on the corresponding side. However, the existing observations indicate that the tonsils are infected in far from all cases. Thus an investigation we performed at meat-packing plants, where we analyzed about 1,800 lymph nodes and tonsils from the carcasses of pigs suspected to be infected by anthrax, established 367 cases of affliction of submaxillary lymph nodes, and only 45 cases of one-sided and bilateral affliction of the tonsils.

Affliction of lymph nodes may be unilateral and bilateral. As an example L. S. Nevskiy (1950) kept 50 pigs with the anginal form of anthrax under observation. He detected unilateral affliction of submaxillary lymph nodes in 47 of them, and bilateral affliction in only 3. Investigation of 136 cases of anthrax in pigs established 87 cases of bilateral and 45 cases of unilateral affliction of submaxillary lymph nodes (Figure 28).

Penetrating through the injured mucosa of the oral cavity, the pathogen travels together with the lymph current to the submaxillary, retropharyngeal, and cervical lymph nodes, which are barriers to the pathogen's further spread.

According to our data, pig blood has preventive properties in relation to anthrax pathogen; this is confirmed by D. M. Teternik's experiments. Conflicting data were obtained in experiments of other authors.

An experiment was performed on rabbits to study the preventive properties of blood serum. Ten-milliliter samples of blood were taken from 10 1-year pigs weighing 80-90 kg. The blood was allowed to settle, serum was obtained, and the latter was injected subcutaneously into rabbits at a dosage of 3 ml per kg body weight. In all, 25 rabbits were used in four experiments (15 experimental and 10 control). All rabbits (experimental and control) were given a subcutaneous injection of day-old broth culture of anthrax microbes at a dose of 0.5 ml 48 hours after injection of pig serum. The control rabbits died of anthrax on the 2d-3d day. Of animals

Figure 28. Afflicted Submaxillary Lymph Nodes

immunized with serum, only two died on the 5th-8th days. Consequently blood serum from healthy pigs has protective properties. This can apparently explain the fact that the pathogen is hardly ever isolated from the blood of pigs stricken with anthrax, and that a septic course is encountered rather rarely. Pigs usually experience the anginal form of disease, with lymph nodes of the head and mesentery affected predominantly.

Course and Symptoms

Pigs suffer the septic (more rarely) and anginal forms of anthrax. The septic form manifests itself very quickly (the signs of disease may be detected on the 2d-3d day), it proceeds acutely, and it usually ends with the death of the animal. The anginal form proceeds over a long period of time as a rule, and it is characterized by affliction of submaxillary, retropharyngeal, cervical, and mesenteric lymph nodes, by arisal of infarctions in the spleen, kidneys, and liver, and by focal lesions in the lungs.

Schlegel (1913) and Frolich (1914) recorded the transitory form of the disease, and Maag (1912) observed the intestinal form. He also noted that this form is encountered very rarely. Out of 154 cases of anthrax in pigs, chronic inflammation was located in 151 cases around the mouth and in local lymph nodes, and sclerotic staining of fatty tissue occurred in only three cases.

Analyzing the published data of many authors (A. V. Katagoshchin, 1910-1913; N. M. Bogdanov, 1910; D. M. Teternik, 1936; L. S. Nevskiy, 1950, etc.), we can say that the anginal form of anthrax is rather widespread in pigs.

The dermal form of anthrax is also encountered; it was described in pigs for the first time by Zschokke in 1887. The dermal form of anthrax is also mentioned by Feldmann (1911), Nieberle (1913), and Stolzer (1914), who found two cases of anthrax in pigs exhibiting numerous hemorrhages on the skin, in addition to affliction of the lymph nodes. These researchers believe that the dermal form of anthrax is actually encountered among pigs much more frequently, but that it is harder to detect owing to the atypical pathoanatomical pattern. However, it should be noted that the dermal form of anthrax has never been diagnosed in our country among animals of this species.

As the diagnostic methods improve, the number of recorded cases of anthrax increase.

According to the statistics, the frequency of different clinical forms of anthrax in pigs is as follows: local form--95 percent of the cases, visceral (internal) forms--5 percent of the cases (of the latter, 48 percent involve affliction of mesenteric lymph nodes, the intestinal form is observed in 23 percent, and parenchymatous organs are affected in 29 percent of the cases).

We studied 1,791 lymph nodes and 380 parenchymatous organs suspected of being infected with anthrax, sampled from a conveyer during inspection of 3,600,000 pig carcasses. Microscopic examination of smears made from the lymph nodes, growth on nutrient mediums, and infection of laboratory animals revealed anthrax bacilli in 367 cases. Integrated pathoanatomical and bacteriological analysis of 136 carcasses confirmed that in all cases, anthrax remained a local affliction. Lymph nodes of the head were afflicted predominantly. Other organs were afflicted in only 10 out of 136 cases: mesenteric lymph nodes in five cases, splenic infarctions in two cases (Figure 29), kidneys in one case (Figure 30), and focal afflictions of the root of the tongue, the thyroid cartilage, and a lymph node of the knee fold [direct translation; possibly the infrapattelar synovial fold] in one case (Figure 31). Bilateral affliction of submaxillary lymph nodes was noted in 87 cases (42.86 percent), and unilateral affliction was noted in 45 cases (22.16 percent).

Anthrax bacillus shadows were detected in the submaxillary lymph nodes in 18 cases; virulent anthrax pathogen culture was isolated from the cervical nodes of the same carcasses as well, anthrax pathogen being detected in 15 of the cases; the shadows of anthrax bacilli and many incidental microflora were found in 21 cases, and tests for anthrax proved negative in 9 cases of thyroids exhibiting pathological alterations. In 14 cases, or 6.9 percent, the anthrax pathogen limited itself to the retropharyngeal lymph nodes (Figure 33). A pure culture was isolated from a lymph node near the ear in one case, and from the thyroid cartilage in another. Mesenteric lymph nodes were afflicted in 5 cases, or 2.46 percent (Figure 34), from which a pure culture of anthrax pathogen was isolated. An altered spleen was found in 2 cases. Hard nodules from a lentil to a hazelnut in size, sharply delimited from surrounding connective tissue, were discovered on the surface of the splenic pulp. When dissected, they appear rather dry, dull, and reddish black in color. *Bac. anthracis* was found within them.

The degree to which clinical signs manifest themselves depends on the form and course of disease. In the acute course of disease, an abrupt increase in body temperature to 42° and more is noted in pigs. The animal is depressed, its movements are sluggish, and its appetite is poor. A sick pig lies on the ground for long periods of time, burying itself in the litter. Sometimes edematous swelling is visible in the neck region (Figure 35). Death occurs in 1-2 days.

Figure 29. Anthrax Infarctions on the Surface of the Spleen

Figure 30. Carcass Kidneys: *a*--afflicted and normal; *b*--smear from a diseased focus in an infected kidney

Body temperature rises insignificantly in the presence of the anginal form of anthrax. A short-lasting increase in temperature to 40.5-41° is possible, but this is not a regularly encountered sign: In some sick pigs, the temperature remains high for 1-2 days, subsequently dropping down to normal. Many animals die in the first day after edema appears in the neck region due to stenosis of the larynx.

Figure 31. Lymph Nodes From the Knee Fold (*a*); *b*--smear from the knee fold lymph node

Figure 32. Focal Affliction of the Thyroid Mucosa

Figure 33. Surface of Dissected Retropharyngeal Lymph Node

Figure 34. Afflicted Mesenteric Lymph Node

Figure 35. Edema in the Submaxillary Region Caused by Anthrax

Sometimes edema gradually disappears, and the animal recovers. Pigs surviving from a case of anthrax may be bacillus carriers.

The disease may proceed in the form of angina when submaxillary or cervical lymph nodes are afflicted. Sometimes the skin in the neck region acquires a bluish-red color; a gelatinous infiltrate appears in subcutaneous tissues of the head and neck, and the animal experiences severe dyspnea and general depression. Breathing and feeding are difficult due to intense swelling of the pharynx. The animal groans, and it chokes when eating. Visible mucous membranes are cyanotic. Very rarely the pathogen remains localized in the lungs. In such cases signs of pneumonia and pleuritis appear.

The intestinal form of the disease is accompanied by digestive disorders, vomiting, and complete loss of appetite; diarrhea or constipation sets in, and feces contain blood. A short-lasting increase in temperature to 40.5-41° is possible.

The disease proceeds chronically rather often. Depending on the place of the pathogen's penetration, lymph nodes in the neck region and the intestine are often afflicted though noticeable clinical signs are not noted. The disease may last for a long period of time, and it is usually diagnosed only after the animal is slaughtered, during inspection of the carcass. The dermal form of anthrax is detected very rarely in pigs. It is characterized by appearance of numerous dark cherry-colored nodules the size of peas and sometimes walnuts on the skin. There are no other signs. Some researchers feel that this form of disease is encountered rather often, but that owing to the atypical clinical pattern and pathoanatomical alterations, it is rarely recorded.

Pathoanatomical Alterations

The anginal form of anthrax often proceeds without manifestation of clinical symptoms. The animal looks entirely healthy, and it is only after slaughter, during careful inspection of lymph nodes and parenchymatous organs, that signs of anthrax affliction are detected. This is why all veterinary technicians subjecting meat to veterinary-sanitary inspection must carefully study the pig carcass and have a good awareness of the pathoanatomical alterations arising in lymph nodes and parenchymatous organs.

Hemorrhagic lymphadenitis may be found in the presence of anthrax, together with affliction of submaxillary, retropharyngeal, and cervical lymph nodes. Other lymph nodes and internal organs are afflicted very rarely. For example *Pasteurella* was isolated from 30 out of 380 analyzed spleens exhibiting different pathoanatomical alterations, and anthrax pathogen was detected in only 2 cases, in splenic infarctions.

Affliction of the indicated lymph nodes may be apparently explained in most cases by the fact that the pathogen enters the body with feed, and if there are wounds or abrasions on the mucous membrane, it readily penetrates through it.

The thyroid glands are afflicted on occasion. These alterations may be unilateral and bilateral, and partial and complete. Focal diphtheric encrustations are visible on the thyroid surface. Foci of hemorrhagic inflammation and ulcers are noticeable in deep-lying tissues beneath these encrustations. Inflammation sometimes spreads from the thyroids to the tongue root, the soft palate, and the epiglottis.

The degree of affliction of submaxillary and retropharyngeal lymph nodes depends on the place of penetration, quantity, and virulence of the pathogen, and the time it is present in the body. Alterations in the lymph nodes become noticeable about 10 days after infection. They affect small areas, the size of a pinhead or pea. It is very difficult to diagnose anthrax at this time. Thus the danger of passing infected carcasses is significant.

If the infectious process continues to develop, the surface of dissected nodes becomes duller, and their color changes to brick red, bright red, or purplish red. Dark red or dark cherry hemorrhages appear on the background of these shades. Gradually the nodal tissue undergoes necrosis, it loses its structure, it becomes loose and brittle, and it crumbles. Such lymph nodes resemble porous limestone. Abscesses, small in some nodes and large in others, are surrounded by connective tissue capsules. Similar changes may be observed when lymph nodes around the ears and in the neck are afflicted. Submaxillary lymph nodes may sometimes reach the size of a chicken egg. Their dissected surface always reveals a constant pathoanatomical pattern.

If the pathogen penetrates to a lymph node through the mucous membrane of the oral cavity, only part of the node or an individual lobe is afflicted. When a massive dose of the pathogen enters a lymph node, the node is observed to turn uniformly bright red. But when the pathogen penetrates from one lobe of the node into others, a cross section of the whole node reveals a mosaic or spotty pattern. Areas of the lymph node into which bacilli had penetrated earlier are more intensively afflicted, and they have a dark cherry color, while places where the pathogen had penetrated later are brick red.

Afflicted areas of a lymph node are separated from intact areas by a reactive zone. In the later stages of anthrax, necrosis affects individual lymph nodes or just some of their lobes.

Owing to erythrocyte breakdown, necrotic areas turn brown in the presence of chronic inflammation. Such alterations are usually observed when tissues undergo healing, and as a rule it is impossible to isolate anthrax cultures from such tissues.

In the next stage of disease the lymph nodes are enlarged and bumpy, and they are often covered by a light yellow or dirty yellow mass.

Hemorrhagic inflammation of a small portion of the intestine, noticeable in the serous membrane, accompanies the intestinal form of disease, which usually has a chronic course in pigs. Sometimes the affected area is rather great, at which time the afflicted segment of the intestine assumes the appearance of a thick, hard tube of dark red color.

The degree of the mucous membrane's affliction varies. Sometimes restricted, swollen, dark red, round foci from several millimeters to 3 cm in diameter are visible. They are located mainly on Peyer's patches, some of them are necrotic, and others appear as a crumbly, greenish yellow or yellowish gray mass. If the necrotic mass is removed, an ulcer with uneven margins is revealed. Scars are visible at the place of a healed ulcer.

Mesenteric lymph nodes that collect lymph from the afflicted portion of the intestine are always altered. The mesentery shows signs of edema in the afflicted region, and its blood vessels are injected. If the disease proceeded sluggishly, the result of productive inflammation may be noted on the periphery of the lymph nodes: proliferation of connective tissue, fusion of intestinal loops to each other and with adjacent organs, encapsulation of inflamed regions, and their occasional necrosis coupled with formation of pus within the capsule.

Only hemorrhagic inflammation in one of the mesenteric nodes and edema of the mesentery may be occasionally detected in the presence of the intestinal form.

According to Bongert (1909), Kirsch (1912), and Elsasser and Siebel (1912), anthrax afflicts the intestine predominantly. Peyer's patches are enlarged, and the mucous membrane is thick. Infarctions from a pea to a hazelnut in size are noted on the surface of the spleen. The spleen, and sometimes liver, are often afflicted (Bongert).

Dommann, Freese, and Wissmann (1909) found lesions in pulmonary and bronchial lymph nodes. Nodules or carbuncles are visible in pulmonary tissue, as are serous-fibrinous exudates in the pleural cavity. The authors indicated that the pulmonary form of anthrax is encountered very rarely in pigs, even more rarely than the septic form.

V. Yu. Vol'ferts (1924) observed anthrax carbuncles in pulmonary tissue and in bronchial glands. Other lymph nodes and organs appeared normal.

There are reports indicating that anthrax pathogen may penetrate into the body through skin. Numerous hemorrhages are visible on the skin in this case.

Diagnosis

An anthrax diagnosis is based on disease symptoms, epizootic data, and bacteriological analyses. Autopsy of carcasses is prohibited in the presence of anthrax. If pathological material must be sampled for analysis, the carcass is placed in an isolated room. Incisions are made of a size permitting sampling of affected tissue with minimum loss of infected material.

If pigs exhibit swelling in the neck region, lymph nodes are taken only from there. In the case of a septic process, the abdominal cavity is opened, and pieces of the spleen, liver, and kidneys and areas of afflicted connective tissue are sampled and analyzed.

An anthrax diagnosis may be made in the presence of latent disease only at the time that the carcass is dressed; all lymph nodes, especially submaxillary ones, and parenchymatous organs must be examined.

Five to ten smears from suspected lymph nodes and organs are stained and inspected with a microscope. If morphologically altered anthrax bacilli and incidental microflora are detected in the smears, the nodes are cut into pieces, one of which is treated with 1 percent carbolic acid solution, and another of which is processed with 70° alcohol for 30 minutes and rinsed several times with physiological solution. Then the material is seeded on nutrient medium. For this purpose afflicted tissue areas treated with 1 percent carbolic solution are grasped with tweezers, they are cut carefully with scissors, and the cut surface is run over the surface of sloped and blood agar in 5 to 10 Petri dishes. The remaining pieces are placed in test tubes containing BEB, placed in a thermostat at 37°, and checked for culture growth in 24-36 hours.

Pieces processed with 70° alcohol are ground in suspension, allowed to stand for 2-3 hours, and then injected into white mice and guinea pigs at a dose from 0.1-0.2 to 0.5 ml. The laboratory animals are kept under observation for 10-20 days. Death of mice means presence of anthrax pathogen in the tested material.

Microscopic analysis of smears does not always reveal typical anthrax rods. Very often the bacilli are morphologically altered (Figure 36)--slighly bent, short, swollen, thickened, and comma-shaped. They may lie as solitary individuals, or in pairs. Incidental microflora may be detected as well--*Pasteurella*, cocci, *Streptococcus*. Experience has shown that is best to prepare smears from afflicted lymph nodes and organs as follows: The suspected node is cut into pieces, the piece exhibiting the greatest degree of affliction is grasped with tweezers, and a cut side of the sample is run over a slide; 5 to 10 such smears are prepared. Then the smears are fixed for 2 minutes and subjected to Gram staining with methylene blue or safranine.

Figure 36. Alteration of the Form of Anthrax Microbes

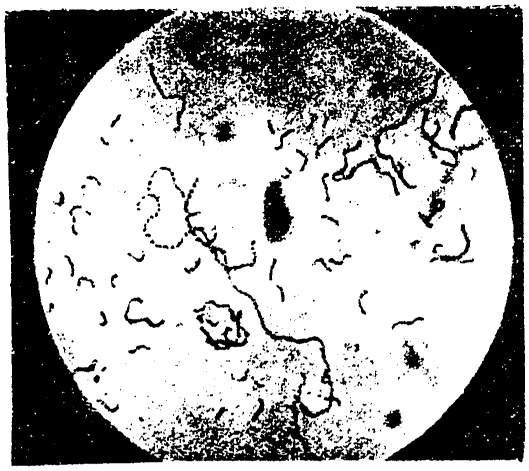

Figure 37. Morphologically Altered Anthrax Microbes (Streptococcal Forms)

It was experimentally established that smears from the submaxillary lymph nodes of pigs suffering the anginal form contain Gram-positive anthrax bacilli and Gram-negative bipolar rods (*Pasteurella*). Growth of both cultures is observed on the first day after the material is inoculated into BEB. When the grown culture was injected into white mice and guinea pigs, the latter died of pasteurellosis, though the initial smears and the cultures (BEB) did contain anthrax bacilli. Therefore it is better to seed suspected material on solid mediums, since even if incidental microflora do grow on them, it is still possible to obtain an isolated anthrax colony, which can easily be reinoculated on BEB and used to infect animals so as to permit a correct diagnosis. Sometimes coccal forms of anthrax microbes are detected in smears (Figure 37).

If the material submitted for analysis is not fresh, it is cut into small pieces, placed in test tubes, covered with broth, and heated at 80° in a water bath for 30 minutes, which kills vegetative forms of incidental microflora, leaving the anthrax spores viable. This material is seeded on blood agar in several petri dishes. The resulting isolated anthrax colonies are inoculated into BEB and permitted to grow, and the broth contents are injected into laboratory animals, followed by isolation of pure anthrax cultures.

The resulting cultures are injected into white mice, guinea pigs, or rabbits. A culture obtained from one sample of pathological material is injected into not one but several mice, since sometimes the strain is so weakened that some immunized mice remain alive.

Differential diagnosis: Plague, erysipelas, and pasteurellosis must be excluded before an anthrax diagnosis can be made.

Points of hemorrhaging of various sizes may be seen on the skin of animals stricken with plague. If the disease had proceeded very quickly, these signs may not be present. Spot hemorrhages beneath the renal capsule, beneath the epicardium, endocardium, pulmonary and costal pleura, and in the mucous membrane of the larynx, epiglottis, stomach, and even the intestine (especially the large intestine) are readily noticeable in the presence of plague. There are usually no necrotic foci in lymph nodes in the presence of plague. When plague is complicated by paratyphus such alterations may occasionally go by unnoticed, but the afflicted areas are usually curdled, soft, and entirely different in appearance from the dry foci seen in the presence of anthrax.

Change in kidney color to dark cherry is typical of erysipelas. Weakly pronounced hemorrhages are evident beneath the renal capsule, or the epicardium, and in the mucous membrane of the stomach and duodenum. Peristatic hyperemia is clearly pronounced.

It is very difficult to differentiate anthrax from pasteurellosis, since their clinical signs are similar in many ways. Often these two disease proceed simultaneously, which reduces the danger of an incorrect diagnosis. Multiple hemorrhages are detected on mucous and serous membranes in the presence of swiftly proceeding pasteurellosis. They are in especially great numbers on the mucosa of the larynx, epiglottis, trachea, pleura, and epicardium. Catarrhal or hemorrhagic inflammation of the abomasum or small intestine is possible. In all cases the accuracy of the diagnosis is determined by laboratory analysis.

Treatment

Modern methods for treating pigs suffering anthrax may be subdivided roughly into specific and nonspecific. Serotherapy is an important method. Antianthrax serum is used against all clinical forms of the disease, and the sooner the better. It is injected subcutaneously or intramuscularly. In severe cases serum heated to 35-37° is infused intravenously; adult pigs are given a single dose of 100-200 ml. Repeat injections are given 1-2 days later depending on the course of disease. Sick animals are first given fractional doses of serum from 5 to 10 ml, and the therapeutic dose is given 20-30 minutes later. Antibiotics are presently enjoying extensive use in the treatment of anthrax in animals.

The use of penicillin against the anginal form of anthrax in pigs was reported for the first time by I. L. Yurkov in 1955. He noted a positive influence by this antibiotic in sick animals. Many researchers report effective treatment of anthrax in pigs with penicillin.

Goldstein (1957) states that during an anthrax outbreak in the state of Ohio (USA) in 1952, 1,614 pigs and 19 head of cattle fell ill; using penicillin at a dosage of 10,000 units per pound of animal weight, the author achieved a positive result. Biomycin, chloramphenicol, and terramycin have approximately the same action. Although some authors have witnessed hopeful results from treating anthrax-stricken animals with penicillin, this treatment method has not yet been developed well.

Certain antibiotics (especially penicillin) are the most effective means for treating sick pigs today. But the question as to the nature of antibiotic action upon the animal body still remains unresolved. Whatever the case may be, it is still better to combine penicillin with serum when treating severe forms of the disease (I. G. Ipatenko, 1969; A. I. Kolobkova, 1953).

Specific anthrax treatment is combined with symptomatic drugs, ones having cardiac action for example. Penicillin is usually administered at dosages from 500,000 to 800,000 units per 100 kg animal weight every 4 hours three times a day, the total dosage varying from 5 million to 12 million units in the entire treatment course, depending on disease severity. The disease may also be treated locally by pricking the zone of affliction, for example the submaxillary space in the presence of the anginal form (300,000 units of penicillin in 0.5 percent novocaine solution).

The therapeutic properties of antianthrax serum and penicillin were studied under production conditions using pigs suffering the anginal form of anthrax (I. G. Ipatenko).

Carcass Inspection at Meat-Packing Plants Before and After Slaughter

Only healthy animals may be slaughtered for meat. A veterinary certificate is issued for every lot of animals sent to the meat-packing plants. A veterinary certificate is issued in the established order for animals delivered for slaughter by private owners. As the lots of animals arrive, the meat-packing plant veterinarian is responsible for checking the correctness of the veterinary certificate, for inspecting all or a sample of the animals, and for taking their temperatures when necessary. When anthrax is discovered in pigs prior to slaughter, the entire lot is quarantined, the animals are meticulously inspected, and their temperatures

are taken. Pigs with a high temperature are isolated and given injections of anti-anthrax serum and penicillin, while animals causing no suspicions are immediately sent for slaughter, after which the submaxillary, retropharyngeal, and cervical lymph nodes and internal organs are carefully inspected. Animals in lots from which individuals stricken by anthrax were isolated or in which animals had died of anthrax while en route are quarantined upon their arrival at the meat-packing plant, they are immunized with antianthrax serum, they are placed under veterinary observation, and their temperatures are taken every day. Three days after immunization, animals with normal temperature are sent for slaughter, after which the submaxillary lymph nodes are carefully inspected. Animals suspected of being sick are isolated and treated with antianthrax serum and antibiotics. They are released from isolation 14 days after their temperature is certified as normal. At this time their submaxillary lymph nodes are meticulously examined at the initial point of anthrax inspection.

Animals to be slaughtered are suspended from special hangers for veterinary inspection and other organs are placed on a table at meat-packing plants not having flow lines for the slaughter and dressing of carcasses, heads, pluck, and spleens. The places of veterinary inspection of carcasses and organs must be well-lit and comfortable. Pig heads are kept with the carcasses until final expert examination following slaughter. The tongue is cut away from the submaxillary space, but it is kept together with pluck. When removed from the carcass, the lungs and tracheae, the heart, and liver must remain in natural communication with one another (as pluck) until completion of their veterinary examination. Associated lymph nodes must also be preserved, with a consideration for focal anthrax-caused alterations that might be diagnosed in the entire course of inspection.

Heads, internal organs, and carcasses are inspected as follows. The outside and inside of the carcass are examined, and special attention is turned to presence of anthrax-caused edema or carbuncles and other pathological alterations. When anthrax is suspected, first the submaxillary, retropharyngeal, and cervical lymph nodes are dissected; if necessary, the rest of the nodes in the carcass are dissected as well. In addition the lymph nodes around the ears are examined, and the tongue and the mucous membrane of the larynx, epiglottis, and thyroid are inspected and probed.

The spleen is subjected to gross inspection, after which the parenchyma is cut and the lymph nodes are dissected. The lungs are subjected to gross inspection and the bronchial lymph nodes (left, right, and middle) are probed and dissected. The liver is examined and probed on the diaphragmic and visceral sides. The stomach, esophagus, intestine, kidneys, and heart are examined, and attached lymph nodes are dissected. The kidneys are extracted from their capsules, and examined and probed. If pathological alterations are discovered the kidneys are dissected; lymph nodes are dissected as well. The intestine is examined on the serous membrane side, and several mesenteric lymph nodes are dissected.

If necrotic foci typical of chronic anthrax are discovered in the lymph nodes of pig heads on the bleeding conveyer, the slaughtering process is immediately halted. The afflicted carcass is removed from the conveyer and transported to the isolation ward. Pathological material is sent to the laboratory for analysis. The bleeding area, the tools, and the hands and work clothing of the veterinarian and workers who had come in contact with this carcass are disinfected, after which the slaughtering process is continued.

If anthrax is discovered after a carcass is dressed, immediate steps are taken to prevent spread of the infection. Further work is halted, and all persons who had come in contact with the anthrax-stricken carcass are determined. Those who had not come in contact with it leave the shop immediately. Then a determination is made as to which carcasses and organs had been in contact with the infected material. Carcasses and organs from animals which had been processed prior to dressing of the sick animal's carcass and which had not come in contact with the suspected material are removed from the shop. These are released without restrictions. Carcasses and organs that had been in contact with the anthrax-stricken carcass, or which are suspected as having been in such contact, are subjected to heat treatment, which must be performed immediately. The afflicted carcass is destroyed. Further slaughtering of the animals is halted when the septic form of anthrax is suspected. Chunks of spleen, altered portions of tissue, and afflicted lymph nodes are removed from the suspected carcass and sent to the laboratory for bacteriological and bacterioscopic analysis. The carcass and all organs are isolated until the analysis results are received. If bacterioscopic analysis establishes presence of anthrax, the carcass is sent out, together with all organs and the hide, for destruction (incineration) without waiting for the results of the bacteriological analysis. All established rules must be complied with at this time. All untraceable products (legs, ears, blood, and so on) obtained from other slaughtered animals and mixed with products from the anthrax-stricken animal are sent out for industrial use, or they are incinerated. After the anthrax-stricken carcass and other slaughter products are removed, the slaughtering shop is immediately subjected to disinfection. Workers perform sanitary cleansing under the instructions and observation of a medical inspector. Other carcasses and slaughter products suspected of having been infected by anthrax bacilli during the production process are subjected to decontamination by cooking right away, but not later than 6 hours after slaughter. If it is impossible to perform decontamination within this time, these carcasses are isolated in a room having a temperature not higher than 10°; the carcasses must be sent out for decontamination, as described above, not later than 48 hours after the time of slaughter. If this is impossible as well, all carcasses and byproducts subject to decontamination are sent out for industrial use, or they are incinerated. Carcasses which had not been exposed to infection by anthrax bacilli during the production process are released without restrictions.

If the results of bacterioscopic analysis of a carcass suspected of being infected with anthrax are negative, it is kept in isolation until the results of bacteriological analysis are in; the veterinarian determines the need for implementing other measures in the shop (disinfection and so on). When the anthrax diagnosis is confirmed by bacteriological analysis, the carcasses and other slaughtering products suspected of being infected by anthrax bacillus are handled as indicated above.

Meat and meat products to be sold at kolkhoz markets and bazaars are subject to mandatory veterinary-sanitary inspection at meat-and-dairy and food control stations. Meat and meat products that had been inspected and marked with a seal of approval outside the market place (at the slaughterhouse, a veterinary station, and so on) and delivered to the market for sale are also subject to mandatory veterinary expert examination at meat-and-dairy and food control stations as well. Internal organs and byproducts delivered separately from a carcass are prohibited from sale. An owner delivering meat and byproducts for sale must simultaneously present a correctly filled-out certificate bearing the signature of a veterinarian (veterinary assistant) and the seal of a veterinary institution, certifying that the animal had been inspected prior to slaughter, that all products had been subjected to veterinary

examination after slaughter in accordance with the established law, and that they originated from a place free of acutely infectious diseases. In this case all carcasses must bear a veterinary inspection seal.

If meat is delivered for sale without a veterinarian's certificate and without a seal, the owner must submit the carcass for inspection, together with the head and internal organs (lungs, heart, liver, spleen, and kidneys). In this case these products are subjected to mandatory laboratory (bacteriological) analysis. The order of sale of these products (without restrictions or after decontamination by cooking) or of their destruction is resolved by a veterinarian depending on the results of the analysis and the veterinary inspection. Entire carcasses or carcasses cut in half or in quarters are submitted for veterinary expert examination at the market place. Meat cut into chunks prior to expert examination at a meat-and-dairy or food control station is prohibited from sale. Carcasses and their parts are subjected to veterinary expert examination according to the existing procedure. At the slightest suspicion of anthrax in lymph nodes or organs, the carcass is placed in isolation, samples are sent to the veterinary-bacteriological laboratory for analysis, and the area is subjected to disinfection.

BIBLIOGRAPHY

1. Bogdanov, N. M., "Trudy 2-go Vserossiyskogo s"yezda vetvrachey" [Works of the Second All-Russian Congress of Veterinarians], Moscow, 1919, p 640.

2. Vol'ferts, V. Yu., VETERINARNYY TRUZHENIK, No 5, 1925, p 10.

3. Vorontsov, V. Ye., VESTNIK OBSHCH. VETERINARII, No 14, 1890, p 122.

4. Ipatenko, N. G., VETERINARIYA, No 3, 1964, p 83.

5. Ipatenko, N. G., "Trudy Gosudarstvennogo nauchno-kontrol'nogo instituta veterinarnykh preparatov" [Works of the State Scientific Control Institute of Veterinary Preparations], Vol 13, 1966, p 297.

6. Ipatenko, N. G., "Trudy Gosudarstvennogo nauchno-kontrol'nogo instituta veterinarnykh preparatov," Vol 15, 1963, p 167.

7. Ipatenko, N. G., VETERINARIYA, No 5, 1968, 38-10.

8. Ipatenko, N. G., VETERINARIYA, No 8, 1969, p 25.

9. Ipatenko, N. G., "Trudy Vsesoyuznogo nauchno-issledovatel'skogo instituta veterinarnoy sanitarii" [Works of the All-Union Scientific Research Institute of Veterinary Medicine], Vol 35, 1970, p 169.

10. Katagoshchin, A. V. "Trudy Vserossiyskogo III s"yezda vetvrachey" [Works of the All-Russian Third Congress of Veterinarians], Vol 1, 1914, p 17.

11. Kolobkova, A. I., "Lecheniye infektsionnykh bol'nykh" [Treatment of Infectious Patients], Moscow, 1950, p 143.

12. Nevskiy, L. S., AGROBIOLOGIYA, No 2, 1953, p 18.

13. Pokshishevskiy, N. A., and Golovin, A. D., PRAKTICHESKAYA VETERINARIYA, No 2, 1931, p 20.

14. Teternik, D. M., "Trudy Vsesoyuznogo instituta eksperimental'noy veterinarii" [Works of the All-Union Institute of Experimental Veterinary Medicine], Vol 15, 1937, p 144.

15. Yurkov, N. L., "Trudy Voronezhskoy oblastnoy nauchno-issledovatel'skoy veterinarnoy opytnoy stantsii" [Works of the Voronezhskaya Oblast Experimental Veterinary Scientific Research Station], 1955, p 4.

16. Bailey, W. W., J. AMER. VET. MED. ASS., No 124, 1954, p 296.

17. Bongert, J., DTSCH. TIERARZTL. WSCHR., No 49, 1948, p 80.

18. Brauell and Barthelmy, REV. VET., No 4, 1890, p 170.

19. Dammann and Freese, DTSCH. TIERARZTL. WSCHR., Vol 72, No 13, 1909, p 635.

20. Elsasser and Siebel, ZTSCHR. FUR FLEISCH.-U. MILCHHYG., No 7, 1912, p 8.

21. Elsasser, ZTSCHR. F. UNF. KR. D. HAUST. SMMG. D. PASTEUR INSTITUTS, No 3, 1925, p 209.

22. Rosca, V., PROBLEME ZOOTEHN. VETER., Vol 9, No 2, 1960, p 61.

23. Schlegel, M., BERL. TIERARZTL. WSCHR., No 41, 1913, p 24.

24. Schmidt, ZTSCHR. F. FLEISCH. U. MILCHHYGIENE, No 13, 1913, p 3.

25. Stein, C. D., Reprint from TEXAS REPORT ON BIOLOGY AND MEDICINE, Vol 11, No 3, 1953, p 534.

VETERINARY-SANITARY MEASURES

The system of anthrax control measures developed and implemented in our country dependably prevents the arisal and spread of anthrax. Veterinary-sanitary measures have great significance within the overall complex of measures applied to places of chronic anthrax infection. These measures are implemented according to plans specific to a given unfavorable point, written by veterinary and medical services as well as other interested departments and institutions, and approved by the executive committees of the soviets of labor deputies.

Many years of practical experience have shown that farms which adhere to a strict veterinary-sanitary regimen while concurrently performing vaccinations and implementing other antiepizootic measures enjoy well-being in terms of infectious animal diseases.

The veterinary well-being of a farm depends in many ways on presence of a complete set of veterinary facilities to support implementation of therapeutic, preventive, and veterinary-sanitary measures (enclosures, a veterinary-sanitary checkpoint furnished with a decontamination unit for transportation and people, isolation wards, a veterinary hospital, a veterinary slaughtering point, and so on), on zoning of the farm's territory into production and administration areas, and its vegetation. Areas containing abundant vegetation have less dust and microorganisms in the air.

Service personnel are permitted to enter the territory of the farm only through a permanently operating disinfection-washing station. Special work clothing and footwear are furnished to service personnel.

Disinfection mats kept constantly moistened with 2 percent caustic soda solution are laid out at the entrances to animal buildings for footwear disinfection.

The veterinary-sanitary condition of production buildings and their surrounding territory is monitored systematically, manure is collected and removed from the farm daily, the condition of treatment plants is maintained under observation, and they are cleaned out regularly.

A sanitation day is held at farms not less than once a month. On this day the entire territory of the farm and all animal and other buildings are cleaned out, contaminated surfaces are washed, and then walls, fences, and posts are whitewashed with freshly slaked lime.

Disinfection is one of the mandatory prerequisites of maintaining sanitary order at farms and preventing animal diseases. Disinfection operations at kolkhozes and sovkhozes are foreseen by veterinary-sanitary plans, and they are conducted under the guidance of veterinary specialists by the farm's manpower or by veterinary-sanitary detachments. Animal buildings are subjected to meticulous preventive disinfection not less than twice a year, while animal buildings at industrial pig and cattle fattening farms are disinfected after the end of each production cycle. Rodents and flies are kept under systematic control.

Zoohygienic requirements of animal care, maintenance, feeding, and watering are complied with strictly in order to raise the natural resistance of the animals. The sanitary condition of pastures, watering places, and cattle drive routes is monitored. Carcasses of wild animals and birds found on pastures are incinerated, and the places where they had lain are carefully decontaminated. Water supply sources are monitored constantly, and steps are taken to prevent their contamination.

The most sensible system, in sanitary respects, for disposing of and decontaminating animal carcasses is to collect and utilize them at plants producing meat-and-bone meal. The work done by such plants in their service zones is aimed at maintaining the veterinary-sanitary well-being of the locale and of the animal farms, and at obtaining meat-and-bone meal to be used as feed, fat to be used for industrial purposes, hides, and other products. The plant's territory and production buildings are separated into two isolated sectors. The raw materials (carcasses, confiscated meat-packing plant products, and so on) are received in the first sector, where they are subjected to initial treatment (dissection of the carcasses, removal of hides, and so on) and, if necessary, incineration; carcasses are subjected to heat treatment in horizontal pressure cookers, raw hides are disinfected and preserved, and finished products are made available in the second sector..

Carcass utilization plants have their own transportation resources (Figure 38) to collect animal carcasses; these trucks travel scheduled routes, or they are dispatched in response to requests by animal owners.

After carcasses are delivered to such a plant, the veterinarian samples the pathological material for anthrax analysis.

Anthrax-stricken carcasses are sent to the carcass incinerator.

All operating and newly opened animal burial and biothermal pits are assigned to regional animal control stations, which draw up veterinary-sanitary cards briefly describing the unique features of each of them.

Animal burial pits are fenced off, and a ditch is dug along the inner perimeter of the fence. A gate is installed to permit entry into the burial pit. Officials appointed by village soviets of labor deputies keep the condition of burial pits under observation.

The carcasses of fallen animals may be autopsied at meat-and-bone meal plants, or in buildings set up for this purpose outside the farm's boundaries, at biothermal pits, and at animal burial pits. Carcasses are delivered to the place of autopsy or utilization by special transportation with a leakproof body.

Figure 38. Specialized Truck Used to Carry Livestock Wastes

At the time of organization of anthrax control measures, special care is taken to comply with the veterinary-sanitary rules associated with procurement, storage, transportation, and processing of raw materials of animal origin. For this purpose veterinary technicians constantly keep procurement agents informed of the order of procuring and transporting raw leather and wool, and they strictly monitor compliance with these rules. Every procurement agent must know that any violation of the established veterinary-sanitary rules may cause people and animals to be infected by this dangerous disease.

Veterinary technicians are obligated to check the veterinary-sanitary condition of places where raw materials are procured. When anthrax arises in population centers and they are declared to be unfavorable points, procurement and transportation of raw materials are halted until the quarantine is removed.

Veterinary technicians are obligated to keep a daily watch over delivery of raw hides and their storage, and to promptly analyze miscellaneous raw material for anthrax.

Every oblast must have two or three disinfection chambers to be used for hide disinfection, and storage space for miscellaneous raw materials of animal origin.

Anthrax control can be successful only when all measures receive the active support of stock breeders and the entire population. This is why the public must be informed. Each person must be aware of the basic information on anthrax, of the

danger of slaughtering animals without a veterinarian's preliminary examination and conclusion, and of the danger of private purchases of meat products that had not been subjected to veterinary inspection, since this could cause infection of people, if the animal turns out to have anthrax. Forced slaughter of animals is performed only at suitably outfitted slaughtering points, in the presence of a veterinary specialist. Sick animals are carried in special transportation.

Special attention is turned to lymph nodes during dressing. Those of animals having anthrax are enlarged, edematous, and hyperemic, and the surface of an incision is dark red in color; the spleen is often enlarged, and its pulp is softened. Other parenchymatous organs are altered as well. Pathoanatomical alterations atypical of anthrax may be encountered; this is why pathological material is mandatorily subjected to bacteriological analysis in all cases.

Pathologically altered organs and edematous tissues are sent to the veterinary laboratory, as are lymph nodes located near the former; submaxillary lymph nodes and surrounding connective (fatty) tissues from pigs are sent additionally. If the internal organs are not delivered together with the carcass of an animal subjected to forced slaughter, lymph nodes and a hollow bone are sent for analysis. In the laboratory, smears are made from the pathological material, conventional mediums (BEB, BEA) are seeded, laboratory animals are infected, and a precipitation reaction is performed when necessary.

Success in anthrax control depends on prompt revelation of the first cases of animal illnes or death. The carcass of an animal killed by anthrax contains a tremendous quantity of microbes. Prompt removal of carcasses and their destruction by incineration is one of the important veterinary-sanitary measures aimed at eliminating an anthrax focus. Animal carcasses are incinerated in permanently installed or mobile carcass incinerators and, when these are unavailable, in trenches dug in the ground.

After every case of isolation and removal of sick animals, all livestock buildings and inside equipment are carefully disinfected. Prior to disinfection, buildings are flushed with disinfectant, and manure and remaining food are cleared away. The manure and feed remnants are incinerated near the infected area with a consideration for sanitary and fire safety requirements.

The following are used for disinfection: calcium hypochlorite or hypochlor solution containing 5 percent active chlorine; 4 percent formaldehyde solution; hot (80°) 10 percent caustic soda solution; 10 percent iodine monochloride solution.

Disinfection is performed three times at 1-hour intervals, using 1 liter of solution per square meter; contaminated surfaces can be processed twice with a 15-25 minute interval only if iodine monochloride is used. After the last treatment, the buildings are closed for 3 hours, after which they are aired.

Ten percent iodine monochloride solution is used at negative temperatures. The solution is applied to the surface at a rate of 1 liter per square meter; the total dose is divided into three applications of 0.3-0.4 liters each. Prior to each application of the disinfectant, the surface is processed with a saturated table salt solution (0.5 liters per square meter), which serves as antifreeze, reducing the freezing point of the disinfectant. Disinfection is performed regularly every

7 days until the final disinfection treatment prior to removal of the quarantine. Disinfection barriers at entrances to buildings are periodically moistened with a Calciumhypochlorite solution containing 5 percent active chlorine. At negative temperatures 10 percent table salt is added to these solutions.

Milk from animals sick with anthrax and animals suspected of being sick is destroyed, while milk from other animals in the quarantine area is processed into butter in the same area, in compliance with the sanitary rules; the butter is subsequently heated at not less than 90° for 3 hours.

Resources indicated on page 190 are used to disinfect milking machines and other milking equipment.

Liquid manure is mixed in a manure collector with dry calcium hypochlorite containing not less than 25 percent active chlorine at a rate of 1 kg calcium hypochlorite for every 20 liters of liquid manure. One day after careful mixing, the liquid is carried to an animal burial pit and buried not less than 2 meters deep. (A. A. Polyakov, 1964).

Special protective clothing and objects used to care for animals are disinfected every day after work is finished at the farm. Metal objects are disinfected in a flame. Tarpaulin, cotton, and felt articles as well as rope, rubber boots and overshoes, brushes, and other objects are decontaminated by being immersed in a 1 percent active solution of chloramine or preparation KhB (sodium parachloro-benzenesulfochloramide, which contains 26 percent active chlorine) for 2 hours at 20° at a rate of 5 liters of disinfectant per kilogram of articles, or in 4 percent formaldehyde solution for 4 hours. Special clothing and other articles are immersed one at a time to insure complete wetting with the solution. Special clothing, harnesses, and animal care objects may be decontaminated in formalin steam chambers. Chamber disinfection entails the use of a veterinary OPPK (combined gas-vapor combustion and formalin-vapor chamber) and medical chambers--the DKP-3 trailer-mounted formalin-vapor disinfection chamber and the UD-2P trailer-mounted disinfection unit.

Articles of clothing are disinfected by the gas-vapor method in an OPPK at 95-100° and relative humidity 84-86 percent for 1.5 hours; the formalin-vapor method requires a temperature of 57-60°, a formalin consumption rate of 250 ml per cubic meter of chamber volume; the loading norm is 3 or 4 outfits or 18-24 kg, and treatment time is 2 hours 45 minutes. Clothing articles are hung loosely in the chamber. Jackets are turned inside-out so that the fur lining is on the outside, felt boot linings and boots are hung with their tops downward, and shoes and slippers are suspended in fishnet bags. The leather parts of yokes, saddles, saddle pads, leather footwear, felt boot linings, and fur clothing are decontaminated in chambers by the formalin-vapor method. The articles are loaded into the chamber on the so-called dirty side, and processed articles are removed on the clean side.

Animals are subjected to external decontamination while the complex of disinfection measures is being implemented at the animal farm. Small farm animals are processed in a special area with its clean half leading to disinfected buildings.

The size of the area depends on the number of animals to be processed simultaneously, the proportion being about 25-30 m^2 per animal. The area is surrounded by a trench 50 cm deep, and it is fenced off. An animal pen, a chute permitting simultaneous

processing of four or five animals, and a drainage pit not less than 2 meters deep are prepared in the dirty half. Animals that had undergone processing in the chutes are driven into a pen in the clean half of the area or into a disinfected building. Decontamination agents, disinfecting equipment, and water and disinfectant containers are located outside the boundaries of the area.

Various veterinary-sanitary and agricultural equipment is used in the processing of animals and land. The LSD-2m disinfection unit (Figure 39), the veterinary disinfection truck (VDM), the truck-mounted disinfection unit (ADA), and the DUK are used in wet external processing of animals with cold and warmed solutions.

The LSD, VDM, and ADA vehicles are used independently or with an additional truck-mounted spraying unit (ARS-12) consisting of a receiver with eight nozzles for attachment of dispensing hoses, and with hose nozzles or brushes.

The following may be used additionally for veterinary processing of animals: various types of fueling trucks and water and oil dispensing units, agricultural sprayers, and portable hand-operated apparatus such as the "Kostyl'" hand sprayer and the "Sever" sprayer (OS-2-1m).

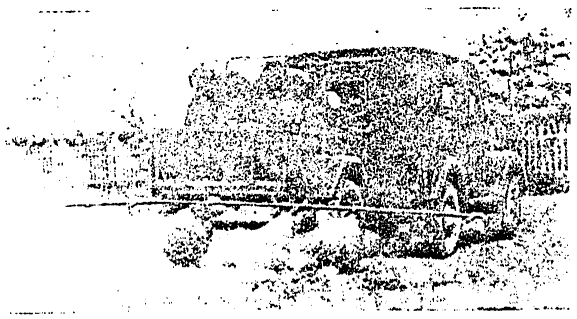

Figure 39. LSD-2m Disinfection Unit

Animals are processed under the guidance of veterinary specialists by workers that had been specially briefed and provided with the necessary protective resources (gloves, overalls, smocks, rubber boots, aprons, oversleeves, caps, safety goggles, and so on).

Prior to starting work, the entire system (reservoir, hoses, nozzles or brushes) is washed off with the working disinfectant. Animals subject to processing are driven into the processing chute (consisting of parallel beams) and isolated there by transverse gates. Processing begins with the head; better wetting of the wool is

achieved in this case if circular movements are made with the brush, first with the grain of the hair, and then opposite it. After hair and skin on the head are thoroughly wetted, all of the rest of the animal is processed.

The following are used in the wet method for external animal disinfection: 8 percent iodine monochloride solution; clarified calcium hypochlorite solution containing 4 percent active chlorine; a solution containing 3 percent hydrogen peroxide and 0.5 percent acetic acid or dichloroglyoxime; a solution of di-tribasic calcium hypochlorite salt or KhB containing 4 percent active chlorine; 2 percent potassium tetrachloriodide solution; an aqueous solution of trichlorisocyanuric acid containing not less than 0.7 percent active chlorine.

The following are used in the directed aerosol method for decontaminating the skin surfaces of animals: a solution of di-tribasic calcium hypochlorite salt or KhB containing 4 percent active chlorine; 2 percent potassium tetrachloriodide solution; a solution containing 3 percent hydrogen peroxide and 0.5 percent acetic acid; 1.5 percent formaldehyde solution. The aerosol is dispensed by either a VDM or an ADA outfitted with an air pump and an aerosol attachment, at a pressure of 0.5 atmospheres. The consumption rates are 20-25 liters per animal with the wet processing method, and 7 liters with the aerosol method.

Animals are processed beneath an awning or in the shade in hot sunny weather. If such shelter is unavailable, the animals are processed twice with an interval of 30 minutes. When the air temperature is below 15°, animals should be processed with solutions heated to 35-40°.

In winter, animals are processed with warm solution (30-35°) in a heated building. After being wetted with disinfectant, contaminated skin areas are additionally cleaned and processed.

Small horned animals and pigs are bathed for 5 minutes in baths containing 7 percent iodine monochloride or 3 percent hydrogen peroxide to which 0.5 percent acetic acid has been added; then they are kept in the clean half of the processing area for 1 hour.

After work, water in the drainage pit is disinfected with calcium hypochlorite at a rate of 1 kg per 20 liters of water; a day later this water is carried to an animal burial pit and buried not less than 2 meters deep. Tools, technical resources, and the enclosure are disinfected. Service personnel undergo personal cleansing; special clothing and footwear is disinfected.

Insects and rodents may serve as a reservoir for anthrax pathogen. Flies inhabiting animal farms and feeding on the mucus, blood, and feces of sick animals also swallow anthrax bacilli, and then they infect feed, water, and other objects with their own feces.

Horseflies and ticks also play an important role in infection transmission. The abundance of horseflies during the hot time of the year and their mass attack of animals make them dangerous spreaders of anthrax. It would be impossible to improve the condition of anthrax foci without eradicating the flies, horseflies, ticks, and other arthropod pests. By organizing insect eradication measures in an anthrax focus entailing the processing of buildings and the spraying and bathing of animals, we can avert their reinfection.

Mouse-like rodents also carry the causative agents of many infectious diseases. A growing number of reports of isolation of anthrax pathogen cultures from wild rodents of various species have been appearing in recent years. Thus according to G. S. Zelenenko (1957), for 4-10 days after feeding on animal materials infected with anthrax, gray rats and house mice are capable of eliminating anthrax pathogen spores together with feces, and spreading them through the environment. M. V. Musayev, F. A. Abushev, and S. Kh. Yuditskaya (1966) isolated anthrax pathogen from a wood mouse. Field rodents can also carry this dangerous disease agent. Moreover field rodents play a role in mechanical spread of this infection: When digging burrows, they transport spores from deep within the soil up to the surface, which creates favorable conditions for infection of agriculture animals on pastures (A. F. Nadzhafov, 1971).

Therefore one of the most important tasks in the complex of anthrax control measures is eradication of mouse-like rodents. Rat extermination in animal buildings requires the use of zoocumarin, ratindan, and phentolacine in food and water traps, as sprays, in the form of toxic foam and sticky substances, and in combination with bacteria pathogenic to mouse-like rodents.

Sources of infection in the environment and vectors can be eliminated only by destroying the anthrax pathogen through disinfection and through insect and rat extermination with modern, effective agents dispensed by the most sophisticated sanitation equipment.

Decontamination of Manure and Waste Water

It would be best to incinerate manure in the presence of anthrax. This is done on-site (in the absence of structures) or somewhere near the farm, in compliance with the necessary veterinary-sanitary and fire safety requirements. The main difficulty in incinerating fresh manure stems from its high moisture content.

The simplest, most easily devised facility for incinerating contaminated manure at animal farms is a trench 75 cm deep and 75-100 cm wide. Metal bars serving as a grate are installed transversely 40-50 cm from the bottom of the trench. Wood is placed beneath the bars. The manure is then piled on the metal bars. If the manure is fresh, it is mixed with straw or wood shavings to hasten combustion.

There are special manure incinerators at railroad disinfection and washing stations and at meat-packing plants.

A. A. Polyakov (1969) proposed a manure incinerator in which manure is dried and incinerated. The furnace, which is made from refractory brick, has a fire box and a drier consisting of three levels, on which the manure is placed. As the wood burns in the fire box, the flames engulf the lowest layer of manure and, passing through the entire manure layer into the smokestack, the flames dry the manure. The latter falls through slits in the lower level of the furnace, or it is pushed through special holes in the furnace. Thus the manure is dried, and it burns well. After infected manure is collected and incinerated, the area from which it had been collected and the working implements are carefully disinfected.

Calcium hypochlorite is used to decontaminate waste water from animal buildings.

A. A. Polyakov (1964) established that dependable decontamination of liquid waste infected with anthrax pathogen requires not less than 7,500 mg active chlorine per liter of unclarified liquid, which is equivalent to 30 gm of 25 percent calcium hypochlorite per liter of this liquid. Calcium hypochlorite is more effective in an acid medium. Therefore if 10 ml unpurified sulfuric acid were to be added for every liter of liquid wastes prior to introduction of the calcium hypochlorite, the latter's action would be more effective, and its dose could be halved.

Before beginning decontamination of liquid wastes, the volume of the waste receptacle and the quantity of liquid it contains are determined in liters. Then the necessary quantity of calcium hypochlorite is calculated, the required amount is weighed out and pulverized, a little water is added, and the resulting watery mixture is poured into the waste receptacle. After careful mixing, the liquid is allowed to stand 24-48 hours, following which it is transported to a specially allocated place.

Liquid wastes from industrial enterprises using animal raw materials are treated in special treatment plants, where they undergo mechanical and biological treatment; prior to being dumped in the sewage system, they are also decontaminated by thermal or chemical methods.

Decontamination by sterilization with live steam is recommended for waste water. There are special autoclaves for this purpose, in which water is heated to 120° by live steam and kept at this temperature for 2 hours.

Waste water cooled to 40° following sterilization is dumped into the sewage system or onto filtration fields.

Among chemical resources, chlorine decontaminates waste water the most reliably. Water is chlorinated with calcium hypochlorite or with bottled chlorine gas.

There are various chlorinating systems used for disinfection of waste water. When anthrax is present, treated waste water is disinfected with chlorine at a dose of 2,000 mg/liter at an exposure time of 6 hours. Following chlorination, the waste water is subjected to chemical and bacteriological control. Given dependable decontamination, the concentration of active residual chlorine in waste water must be not less than 35 mg/liter 2 hours after treatment.

Sludge from the settling tank is incinerated or carefully mixed with dry calcium hypochlorite at a ratio of 1 part hypochlorite to 4 parts sludge, which is then buried.

Untreated waste water is decontaminated with dry sodium hypochlorite at a rate of 1 part hypochlorite to 4 parts contaminated water; the latter is then held for 6 hours.

S. A. Michko (1967) recommends liquid chlorine for decontamination of waste water from biological industry enterprises producing biological anthrax preparations. In this case the waste water is decontaminated after initial treatment. Chlorination is performed in contact settling tanks with chlorinators at a rate of 500 mg active chlorine per liter of waste water; the dose is regulated on the basis of residual chlorine. Exposure time is 2 hours. The concentration of active residual chlorine in the waste water must be not less than 35 mg/liter 2 hours after chlorination.

Sanitary and bacteriological control of the effectiveness of waste water chlorination entails determining active residual chlorine every 2 hours and subjecting waste water to bacteriological analysis not less than once every 10 days after chlorination, simultaneously with regular determination of active residual chlorine.

Research conducted at the All-Union Scientific Research Institute of Veterinary Sanitation (S. A. Michko, 1972) established the possibility for using gamma-rays to decontaminate waste water infected by anthrax pathogen at enterprises of biological industry. A 1.9 mrad dose sterilizes waste water exhibiting maximum density of bacterial and organic contamination.

Decontamination of Water Basins.

The simplest method for decontaminating water basins containing anthrax pathogen is chlorination with calcium hypochlorite, chloramine, and chlorine dioxide. Chlorine-containing substances are dosed on the basis of the concentration of active chlorine. The dose of chlorine required for decontamination of water is set such that excess chlorine would be present after its absorption by the water.

Prior to chlorination, determinations are made of the volume of water in the basin to be decontaminated, the required dose of active chlorine in miligrams per liter of water, and the total quantity of disinfectant needed for the given volume of water.

Disinfection of water basins containing anthrax pathogen requires 200 mg chlorine per liter of water. A calcium hypochlorite solution containing 5 percent active chlorine is prepared for this purpose. The water basin is chlorinated with this solution at a rate of 4 liters per cubic meter of water (200 mg/liter). In this case chlorination of 100 liters of water would require 96 gm, and 1,000 liters would require 960 gm of calcium hypochlorite containing 25 percent active chlorine. If the chlorine-containing substance has a higher active chlorine concentration, less of it would be required. To chlorinate a water basin, a weighed quantity of calcium hypochlorite is carefully mixed in an appropriate container with a small quantity of water until a pasty consistency. This mixture is then poured evenly onto the surface of the water basin in small batches. If possible, after the calcium hypochlorite solution is added to the water basin, the water within it is mixed. Chlorination time is 12 hours. After chlorination, if the water basin is small the water is gradually drained from it into a prepared trench. The bottom of the water basin is disinfected, and it is left unfilled with water for the summer for the purposes of insolation.

Disinfection of Soil

Soil decontamination is one of the complex and important elements of the complex of veterinary-sanitary measures aimed at eliminating anthrax. Organic substances in the soil hinder penetration of disinfectants into the deep layers. The more organic matter the soil contains, the faster the mineral and organic particles of the soil absorb chemical agents, arresting them in the superficial layers. Therefore when decontaminating soil of such composition, it must be mixed with the disinfectant especially carefully.

Soil is decontaminated by chemical and thermal means. For this purpose it is first irrigated with a calcium hypochlorite suspension containing 5 percent active chlorine at a rate of 10 liters per square meter. Then the soil is dug up to a depth of not less than 25 cm, mixing it with dry calcium hypochlorite at a 1:3 ratio, and it is moistened with water. The superficial layer of soil, up to 3 cm deep, is disinfected with 4 percent formaldehyde solution at a rate of 5 liters per square meter; disinfection time is 24 hours.

Sh. Kh. Kharisov, Kh. Kh. Abdullin, and L. N. Ryzhov (1969) designed the KVI unit for thermal sterilization of soil infected by anthrax pathogen. During the unit's work, the soil is gradually heated to 350° at a depth of 5 cm, to 250° at 10 cm, and to 100-120° at 15-20 cm. Heating of the soil for 10-14 hours kills anthrax pathogen spores to a depth of 20 cm.

A. A. Polyakov and G. D. Volkovskiy (1974) suggest OKEBM gaseous mixture for disinfection of plowed soil to a depth of 40 cm; disinfection is performed beneath PK-4 polyamide film. Decontamination is achieved at temperatures not below +5° at a moisture content from 1 to 3 percent. The rate of gas mixture consumption per square meter is 1 kg with an exposure time of 5 days, and 0.5 kg with an exposure time of 10 days.

V. I. Pilipenko (1974) suggests using methylbromide for these same purposes. Decontamination is achieved with a gas consumption rate of 1.5 kg per square meter with an exposure time of 7 days, 1 kg with an exposure time of 10 days, and 0.5 kg with an exposure time of 20 days.

Measures Implemented Prior to Lifting of a Quarantine

After the quarantine period--that is, 15 days after the last death or recovery of an animal stricken with anthrax, follow-through measures are implemented at the quarantine station.

Jointly with a representative of the local soviet of labor deputies, a veterinarian checks the compliance of the antianthrax measures with veterinary law, and he implements the follow-through measures. For this purpose all animals that had contact with sick animals or ones suspected of having been infected by anthrax are driven out of the buildings into a pen, where they remain until the decontamination measures are concluded and the buildings are aired following final disinfection.

At first the buildings are flushed with solutions recommended for disinfection in the presence of anthrax, and then they are subjected to meticulous mechanical cleaning. Manure, feed remnants, and trash are incinerated. The wooden floors of stalls in which sick animals had been present are removed and burned (if this had not been done earlier, following removal of sick animals), the upper layer of ground is disinfected down to the clay base, and then it is transported to an animal burial pit in a sealed container and buried not less than 2 meters deep. Inexpensive wooden equipment is burned. After the new floors are laid, the buildings and objects of animal care are carefully disinfected.

Liquid manure contained in the receptacles of anthrax-stricken animal yards are disinfected with dry calcium hypochlorite (p 183). Infected soil areas around the animal yard and animal walkways are also disinfected (see above).

Animals are subjected to meticulous external processing (p 180) and led into a building that had been aired after disinfection.

All transportation resources and other equipment that had come in contact with the infected material are disinfected.

After special protective clothing and footwear worn by workers that had implemented the follow-through measures are decontaminated, a document indicating all sanitary-disinfection measures implemented during the time of the quarantine is drawn up.

Measures Implemented Aboard Transportation in Response to Appearance of Anthrax or When Conveying Infected Raw Materials

When animals brought in for transportation are discovered to include ones having or suspected of having anthrax, the entire lot is detained and subjected to meticulous veterinary examination and thermometry, after which measures foreseen in the anthrax control manual are implemented.

Forage with which sick animals and ones suspected of illness had come into contact is incinerated. All articles that had come in contact with the stricken lot of animals are disinfected, and inexpenseive tools are burned. The loading areas, platforms, pens, and other places where the animals had stood are carefully cleaned and disinfected, and manure and trash are incinerated.

If animals are discovered to have anthrax while en route, the entire lot of animals is detained and unloaded. Sick animals and ones suspected of illness are isolated and treated. The remaining animals are immunized with antianthrax serum and quarantined. Carcasses are incinerated.

Rail cars, ships, and barges that had contained sick animals or raw materials of animal origin infected by anthrax pathogen are classified, in relation to the nature of veterinary-sanitary processing required, as being in the third category; therefore they and all associated equipment, manure, and feed remnants are sent to a disinfection-washing station for veterinary-sanitary processing. The doors of rail cars and the hatches of vessels and barges are closed and sealed, and their outer surfaces are disinfected. Labels bearing the inscription "Routed for Disinfection" are glued to both doors of a rail car, or the inscription is written on clearly with chalk. It is also stated in the freighting documents for rail cars and in special instructions for vessels and barges that the rail cars, vessels, or barges are routed for disinfection and are traveling in category 3, and a note as to equipment contained in the rail car is written on the rail car sheet.

If rail cars and vessels fail to arrive at the disinfection and washing station within a set period of time, the station chief organizes a search for them and makes the necessary arrangements for their processing.

When rail cars arrive at the disinfection and washing station, first the inside surfaces of the walls, the floors, and manure as well as all equipment inside the cars are irrigated with disinfectant. Then manure is carefully cleaned off of all surfaces, and manure and inexpensive equipment are burned. After this the cars are

disinfected together with all of the equipment they contain: The outside and inside are processed with clarified calcium hypochlorite or hypochlor solution containing not less than 5 percent active chlorine, or by 4 percent formaldehyde solution, at a disinfectant consumption rate of 0.5 liters per square meter.

Thirty minutes after disinfection the inside and outside of the rail cars are carefully rinsed with hot water (60-70°) dispensed as a stream at not less than two atmospheres of pressure.

Contaminants that cannot be flushed off are removed with a scraper, brush, or broom. Then the surfaces of the rail car are washed once again until the draining water is completely clear.

After the rail cars are washed, the inside is disinfected once again at a decontaminant consumption rate of 1 liter per square meter. The decontaminant is applied to the surface four or five times as a fine spray. Then the doors of the rail car are closed, and the outer surface of the rail cars, the footboards, brake platforms, and undercarriage are disinfected at a solution consumption rate of 0.5 liters per square meter. Thirty minutes after disinfection is finished, the rail cars are released from the station.

Waste water collected separately from category 3 rail cars during their disinfection and washing is decontaminated by autoclaving at 120° for 2 hours, or by chlorination coupled with preliminary coagulation. After neutralization of the chlorine, the disinfected waste water is dumped in the general sewage system.

The order of veterinary-sanitary processing of vessels and barges is similar to rail car processing. Holds cleared of manure and trash are disinfected twice at a 2-hour interval. One hour later surfaces undergoing decontamination are rinsed with hot water.

Rinse water collected after vessel processing is drained into liquid collectors installed aboard the vessel, and then pumped into special water treatment vessels.

The winter temperature of processed surfaces must be above 0°. For this purpose rail cars and vessels are washed and disinfected with hatches and doors closed.

All work associated with cleaning, washing, and disinfecting rail cars and vessels is done by specially trained people in the presence of a veterinary inspector. After work is finished the special clothing is disinfected and stored in individual lockers at the decontamination counter.

All articles and tools used to clean and wash the rail cars and vessels are decontaminated daily after work, and stored in a separate space.

Airplanes that had carried animals or animal raw materials infected by anthrax are decontaminated with formaldehyde applied as an aerosol. After cleaning, the airplane is disinfected with an aerosol consisting of 40 percent aqueous formaldehyde solution at a consumption rate of 40 ml per cubic meter of internal airplane space. Air temperature outside and inside the airplane must not be less than 12-15°, and exposure time is 24 hours. If the outside air temperature is below 12° the airplane is heated prior to disinfection, so that the air temperature inside it would be within 25-30°.

Formaldehyde aerosol is produced with a TAN turbulent aerosol attachment and a compressor generating an air pressure of 4-4.5 atmospheres and having a delivery of not less than 30 cubic meters of air per hour. The aerosol stream is directed along the cabin aisle, and through inside hatches into the airplane's cargo compartment. The airplane is sealed off prior to introduction of the aerosol. After the disinfection time expires, gases are removed from the airplane by fanning the cabin, the passenger compartment, and all cargo compartments with blower-equipped heaters for 24-48 hours.

Motor vehicles that had carried anthrax-infected animals or raw materials of animal origin are decontaminated in a specially prepared area on instructions from the veterinary inspector. Before disinfection, the vehicle's cab is irrigated with disinfectant, after which dirt is removed mechanically. Wet disinfection is performed with a hypochlorite solution (5 percent active chlorine) or a 6 percent formaldehyde solution containing 0.5 percent detergent. Wet disinfection is performed twice with solution heated to a temperature of 50-60°.

Measures Against Anthrax at Meat-Packing Plants and Dairies

When animals with anthrax are discovered at the animal base of a meat-packing plant, veterinary-sanitary measures are implemented in accordance with instructions of the USSR Ministry of Agriculture in the same way as at any other livestock complex.

Permission to slaughter cattle and small horned animals for meat is granted 3 days after immunization with antianthrax serum, assuming normal body temperature and absence of signs of illness; animals immunized with vaccine may be slaughtered 14 days after their immunization and if they exhibit no reaction to immunization, or signs of illness.

Slaughter of pigs is permitted at a sanitary slaughterhouse when body temperature is normal and clinical signs of illness are absent.

Animals that had suffered anthrax may be slaughtered 14 days after complete recovery. Prior to slaughter, the animals are subjected to external processing with decontaminants.

A quarantine is removed from an animal base 15 days after the last death of an animal or isolation of one stricken with anthrax, and after final disinfection is performed.

When an anthrax-infected carcass is discovered on a conveyer, the production process at the meat-packing plant is immediately halted, and it is resumed only with the permission of the veterinary service, which organizes and implements the necessary veterinary-sanitary measures.

The carcass of the anthrax-infected animal is incinerated together with all organs; the carcasses of healthy animals and all untraceable products (legs, ears, udders, and so on) that had come in contact with slaughtering products from the anthrax-infected animals are also incinerated. The hide of the anthrax-stricken animal is sought out from the common pile of hides and burned, while all other hides of this lot are disinfected.

Other carcasses and slaughtering products suspected of being infected by anthrax pathogen during the production process are immediately decontaminated by cooking, not later than 6 hours after the moment of slaughter (prior to spore formation), given normal storage conditions. If it is impossible to decontaminate the carcass and organs within this time period, they are isolated in a building having a temperature no higher than -10°. They must be sent out for decontamination, as described above, not later than 48 hours after the animal's slaughter. If not, all carcasses and byproducts subject to decontamination are surrendered for industrial utilization or incineration. Carcasses that had not come in contact with slaughtering products from the anthrax-infected animals are released without restrictions.

Meat and meat products are decontaminated by cooking in chunks weighing not more than 2 kg and up to 8 cm thick, in uncovered pots for 3 hours after boiling begins, and in covered pots at a steam pressure of 0.5 atmospheres for 2.5 hours.

All workers of the meat-packing plant who had come into contact with animals stricken with anthrax, or with products originating from these animals, must undergo personal cleansing.

Raw material and production shops are decontaminated in the following order: Buildings and equipment of the animal base, animal walkways, and other shops of the meat-packing plant in which a consignment containing sick animals had been held or through which such animals had been driven are prepared for disinfection, as are facilities that had handled the slaughtering products of such animals (surfaces are sprayed with decontaminating solution, degreased, and cleaned); buildings, shop territory, and equipment are disinfected; building and equipment are subjected to final disinfection.

Before disinfection, contaminants and raw material residues are removed from the shops. Tools are stored in a separate building for disinfection. First all buildings and large metallic tools and equipment (carts, vats, suspended buckets, conveyers, and so on) are sprayed with hot 10 percent caustic soda solution. One hour later the walls, floors, and all equipment are washed with hot 1-2 percent (dempa) solution and cleaned with a stream of pressurized warm (40-45°) water or, if necessary, with scrapers and stiff brushes. The collected trash and manure is carried away in a special container and burned in a trash incinerator. Then the buildings are disinfected a second time with one of the following solutions: hot 10 percent caustic soda solution, calcium hypochlorite or hypochlor solution (5 percent active chlorine), 4 percent formaldehyde solution. Disinfection is repeated 1 hour later, expending 1 liter of solution per square meter of space each time.

After cleaning and decontamination, small metal and wooden tools are immersed for 2-3 hours in a vat filled with calcium hypochlorite solution containing 5 percent active chlorine. The parts of dismantled production equipment prepared for disinfection (hydrometers, cutters, mixers, and so on) are sprayed with the same solution dispensed as a directed aerosol with TAN and PVAN aerosol attachments, while small parts are immersed in disinfectant for 2-3 hours, or they are decontaminated by boiling for 30 minutes. If machinery cannot be dismantled easily, disinfectant is poured in, the machine is turned on, and it is run until all of its parts have been washed with the solution. Hypochlor preparation (5 percent active chlorine), which is the least corrosive, is the best agent for disinfecting production equipment.

Following disinfection, machines and individual parts are rinsed with water from a hose, wiped dry, and coated with fat to avoid corrosion. Valuable equipment and drive belts are carefully wiped with rags moistened with 4 percent formaldehyde solution. Used rags are burned. Knives, (musaty), sheaths, choppers, buckets, and so on are boiled for 30 minutes or autoclaved for 1 hour at two atmospheres of pressure.

Clothing and packaging material are decontaminated in formalin-vapor disinfection chambers.

Prior to disinfection, the temperature of refrigerator compartments is lowered to 1-2°, after which the compartments are disinfected with 10 percent iodine monochloride solution, twice with a 15-25 minute interval, or with 4 percent formaldehyde solution.

Following disinfection for an appropriate time period and disinfection quality control, all surfaces and equipment are rinsed with water, and all buildings are whitewashed with chalk or lime solution. After this the meat-packing plant may be placed back into operation.

If a dairy receives milk from a quarantine point, the milk is processed into pot butter, and the building, equipment, tools, and special clothing are carefully disinfected twice with a 30 minute interval, using clarified calcium hypochlorite or the di-tribasic salt of calcium hypochlorite (DTSCH) containing not less than 5 percent active chlorine in solution; 3 percent chloramine solution or 2 percent DTSCH solution activated by ammonium sulfate or chloride (5 gm per liter of solution); hot 10 percent caustic soda solution; 15 percent Perhydrol solution. The solutions are used at a rate of 2 liters per square meter of building space with a 2-hour interval. After the second treatment, all equipment is rinsed, and the building is aired.

Pasteurizers, pipelines, tanks, refrigerators, vats, and milk tank trucks are decontaminated with steam dispensed for 20 minutes at an excess pressure of 1.5-2 atmospheres, and with hot disinfecting solutions (65-70°): 10 percent Perhydrol solution; 2 percent activated DTSCH solution; 4 percent activated chloramine solution for 30 minutes. A quantity of disinfectant sufficient for normal circulation is fed into the milk processing and transporting system. Following disinfection, the system is rinsed with hot water for 15-20 minutes. Filter materials, dirt, and sludge from centrifugal separators are burned in a specially allocated place. Metal and glass containers (bottles, jars, and so on) are immersed in 10 percent hydrogen peroxide solution, or 2 percent activated DTSCH solution, or 4 percent activated chloramine solution for 2 hours.

Following disinfection of the buildings and equipment, the special clothing, rubber boots, and gloves of the workers are decontaminated in disinfection chambers, or they are wetted with disinfectants.

Maintaining the Veterinary-Sanitary Well-Being of Raw Hide Plants, Bases, and Warehouses

Within the overall complex of veterinary-sanitary and preventive measures against anthrax, an important place should be given to strict veterinary surveillance over

the procurement, storage, transportation, and initial processing of raw hides, and over the organization and maintenance of enterprises storing and processing raw materials of animal origin.

Raw hide and wool have long played a major role in the spread of a large number of infectious diseases, anthrax especially.

A skin removed from the carcass of an animal killed by anthrax or subjected to forced slaughter is a dangerous source of infection, since vegetative forms of the pathogen form spores within 2 hours after the skin's removal. These spores are usually located near blood vessels on the side facing the subcutaneous fatty tissue, in the papillar layer, and directly beneath the epidermis.

Moreover anthrax spores may land on the wool surface of a hide together with feces, urine, and blood during forced slaughter of sick animals or during the removal of the skins of dead animals.

It has been established that anthrax pathogen spores attach themselves to wool, even after settling on it by chance, and that at 20° they may germinate into vegetative forms, proliferate, and once again form spores, infecting the wool more and more. That anthrax bacilli can develop on hide scrapings has been demonstrated as well. The quantity of anthrax bacilli in raw hides may grow by a factor of 1,000 and more (Gegenbauer, 1920; Ye. S. Cherkasskiy, B. P. Chirkov, 1960).

L. L. Kukharkova (1930) and G. S. Zelenenko (1957) report that in 50-85.5 percent of the cases, typical anthrax pathogen cultures pathogenic to laboratory animals were isolated from raw hides subjected to bacteriological analysis and having a positive precipitation reaction to anthrax.

According to R. I. Rassovskaya and N. E. Agadzhanov (1933) preservation by fresh-drying and wet-salting does not reduce the virulence of anthrax bacilli. Addition of antiseptic in an effort to preserve raw hide does not kill spores (L. Ye. Naymushina, 1956; S. A. Kaspar'yants, M. S. Lyuksemburg, 1971). Observations by D. K. Zabolotnyy (1927), N. F. Gamaleya (1940), and Ye. I. Minkevich (1945) indicate that anthrax pathogen spores can survive for years even in tanned hides.

Wool, bristle, and hair are also a significant factor in the transmission of anthrax pathogen to animals and man (A. G. Birger, L. A. Korobitsina, V. A. Nabokov, 1927).

Sufficiently dependable protection of workers in raw hide industry against infection by anthrax at work has now been achieved in the USSR owing to implementation of planned veterinary-sanitary and preventive measures.

However, because the USSR gets large quantities of raw hides from Asian and African countries with an anthrax problem, and because such hides are transported by rail to raw hide plants and bases deep within our country without analysis, a possibility for spreading infection is created and the danger of infecting people with anthrax, both those working in leather production and those living near such enterprises, arises. In this case hides subjected to dry preservation are infected most often.

All of this makes strict veterinary-sanitary surveillance over the storage and initial processing of raw hides at enterprises necessary. A large amount of dust

that contaminates the air and settles on surfaces forms at such enterprises during the unpacking, sorting, and transportation of raw hides. An especially great deal of dust is formed during work with hides preserved by dry salting.

Our survey of plants and bases subjecting imported raw hides to initial processing showed that the air of shops at raw hide plants and bases is significantly contaminated by microorganisms (up to 200,000 microorganisms per cubic meter of air) and that it contains spore-producing microflora; also, 5.8 percent of the air samples taken from shops involved in the initial processing of imported raw hides contain anthrax pathogen spores. In this case 50 percent of the analyzed samples of dust settling on surfaces in the sorting and quarantine divisions contained viable anthrax pathogen spores. The soil of territory around raw hide plants turned out to be infected by anthrax pathogen spores in 15 percent of the cases.

The results of our bacteriological analysis of raw hides, the air and surfaces of buildings, and the soil on territory around raw hide plants and bases doubtlessly confirm the fact that work at such enterprises is associated with a real possibility for spreading anthrax pathogen and infecting people with this pathogen. And if cases of job-related anthrax are rare among people in the USSR, this fact attests only to the dependability of STI vaccine, with which laborers and white collar workers of plants and bases are immunized mandatorily. However, absence of illness among immunized laborers and white collar workers cannot be an indication of well-being in relation to anthrax at raw hide processing plants and bases. In fact, enterprises processing imported raw hides infected with anthrax may be permanent foci of this disease, since dust formed during the processing of infected hides contains viable anthrax pathogen spores.

General Veterinary-Sanitary Measures

The sanitary well-being of enterprises responsible for the storage and initial processing of raw materials of animal origin depends in many ways on how fully veterinary-sanitary requirements on master plans, buildings, structures, and production processes involved in the processing of infected raw materials are met during planning. This is why the veterinary service must maintain preventive sanitary surveillance, beginning during the planning of enterprises storing and processing raw materials of animal origin, as well as during the selection and allocation of lots for construction of such enterprises. It is very important to divide the territory strictly into administrative and production zones. The production zone is enclosed by a fence not less than 2 meters high. This zone is the site of the isolation area for raw materials suspected of being infected with anthrax, disinfection chambers, the trash incinerator, the salt decontamination kiln, waste water settling tanks, and decontamination facilities, all of which should be located in an isolated area of the territory.

The enterprise's entire production territory is paved with asphalt. Its boundaries are edged by a safety strip containing a gutter communicating with the sewage system and the settling tanks.

Two-way traffic is undesirable within the production zone. As motor vehicles leave this territory, they must pass through a decontamination center operating in winter and summer.

The warehouse and storage office buildings must have sufficient area to permit separate storage of slaughterhouse materials, nontraceable raw materials that have already been analyzed for anthrax, and raw materials subject to analysis, as well as a place to store wool and other raw materials. A second exit is foreseen in these rooms for transfer of raw materials to connecting rooms handling subsequent stages of the production process (an exit into a sorting room for raw hides, and one into drying, grating, and pressing rooms for wool); an isolated area is also foreseen on the territory of the warehouse consisting of two divisions, one for storage of raw material suspected of being infected by anthrax prior to receipt of the analysis results, and raw materials having a positive precipitation reaction to anthrax. A raw material disinfection chamber is adjacent to the isolated area. There is a special place for workers--a dressing room equipped with a shower and individual lockers for separate storage of clean street and work clothing, and at a decontamination center. Workers servicing the isolation area, the raw material disinfection chamber, the trash incinerator, and the salt roasting kiln have a separate decontamination center.

The walls and ceilings of warehousing space must be accessible to cleaning, washing, and wet disinfection. Floors and drain gutters must be watertight, and inclined to permit drainage of liquid into special concrete receptacles for subsequent decontamination.

Spent salt and all waste and trash are collected daily in metallic containers, which are sent to the incinerator as they are filled.

The warehouse yard must have a level surface, and it must be kept clean. Disinfection mats wetted with calcium hypochlorite solution (5 percent active chlorine) are placed before building entrances.

Special attention is turned to the veterinary-sanitary condition of tents set up by rayon procurement offices at marketplaces. They are pitched away from the market stalls, a safety zone not less than 50 meters wide is maintained around them, and the territory is fenced off. Spaces for receipt and storage of raw materials for a one-day period are set up in a tent.

Raw hides and furs, wool, and hair are stored on racks in warehouses in such a way as to easily permit regular cleaning and disinfection. Not less than twice a year the warehousing space is carefully cleaned and subjected to preventive disinfection. Isolation areas, the sites of disinfection chambers, and tents belonging to rayon procurement offices are cleaned and disinfected every time raw materials are forwarded elsewhere.

Flies and mouse-rodents are kept under control in summer.

Veterinary technicians have an obligation to maintain daily surveillance over incoming raw hides and their storage, and to promptly analyze miscellaneous raw materials for anthrax. Samples must be taken for analysis only under the observation of veterinary personnel. Every sample sent to the laboratory is accompanied by a certificate indicating the number of the stack or bundle from which the sample had been taken. The necessary hide can easily be found as a result. Until the laboratory results are reported, movement and sorting of the raw hides is not permitted.

All hides must be stamped. Miscellaneous raw hides are stamped only after being analyzed for anthrax in the presence of a veterinary technician. Raw hides procured from a slaughterhouse are stamped right at the slaughterhouse or at a meat-and-dairy control station following inspection of carcasses by a veterinarian. A rubber stamp designed by L. Ye. Naymushina is the best. It has a spring-driven mechanism producing a clear impression on uneven hide surfaces. The stamp must be stored in a particular place under lock and key.

Veterinary certificates are a confirmation of the safety of raw hides. However, precise accounting and proper stacking of raw hides must be organized at warehouses in order to guarantee the sanitary safety of raw materials released on the basis of veterinary certificates. With such organization, on inspecting documents accompanying raw materials entering the warehouse and checking for their presence in the stacks, the veterinarian is able to fully guarantee the sanitary safety of raw hides released from the warehouse.

While stiff requirements are imposed on domestically produced raw hides, the veterinary inspection office must relate even more strictly to imported hides. This is why it is very important to intensify veterinary-sanitary surveillance over the initial processing of imported raw hides, as well as over the organization and equipment of enterprises processing this raw material.

Imported raw hides are subjected to initial processing at raw hide plants and bases, at which a possibility exists for allocating special shops for this purpose, completely isolated from other buildings of the plant or base. Territory intended for processing of imported raw materials is isolated by a safety strip containing a gutter communicating with the sewage system, so that rain and thaw water would not enter the plant territory.

The rail bed in the quarantine division's unloading area intended for imported raw hides has an isolated concrete surface with a gutter communicating with the sewage system.

Building windows do not have sills, and window sashes are built flush with the inner surfaces of the walls. The windows do not open.

The shop intended for initial processing of imported raw hides must be outfitted for separate processing of dry- and wet-salted raw hides. For this purpose the shop has quarantine sections, places for taking hide samples for anthrax analysis, areas for temporary storage of raw hides prior to receipt of the laboratory results--the pre-isolation area, which adjoins to the hide disinfection chamber and the isolation area; places for storage of analyzed raw material prior to sorting; sorting sections; sections for storage of sorted raw materials prior to release to the consumer; personnel areas--checkpoints leading to the dressing room, shower, and washroom, areas for washing and disinfection of special footwear, and areas for removal of dust from special work clothing, and for its drying. All areas in the shop must be subdivided by partitions, and they should communicate only through wide sliding doors.

The Order of Initial Processing of Raw Hide

To prevent two-way traffic, production areas intended for initial processing of raw hides are arranged with the flow of the production process. Incoming raw hides are

unloaded from rail cars or trucks in the quarantine section. From here, the bundles are moved to the shop as the need arises; here they are unpackaged, and samples are taken for anthrax analysis. After samples are taken, the bundles are sent to the pre-isolation area, where they are stacked and held until the laboratory results are in. Then hides having a negative precipitation reaction are sent to the next area, where they are stored until sorting. Raw hides are sorted in a separate area. And it is only after this that they are moved to their place of storage prior to release to the consumer.

If hides with a positive or doubtful precipitation reaction are discovered in the bundles (piles), they are sent to the isolation area, where not less than three samples are taken at different places on each hide for analysis. Hides that exhibit a positive reaction to anthrax a second time are burned under the control of a veterinarian, and all of the remaining hides in the particular bundle (pile) are disinfected.

Imported raw hides having a negative reaction to anthrax must not be stored together with domestically produced hides.

Imported raw hides and imported wool are conveyed at the enterprise by motor transportation specially allocated for this purpose. Use of this transportation to convey food and forage is prohibited.

Dust Control Measures

First of all it would be suitable to concentrate all dust-producing operations in separate areas outfitted with an efficient ventilation system; all dust-producing operations should be mechanized, the production areas should be vacuum-cleaned regularly, and workers should be supplied with individual dust control resources.

Our research showed that an especially great deal of dust is created during the sorting of raw hides, their unpackaging, and sampling for analysis. Therefore sorting areas and--in shops involved with initial processing of imported raw hides--the sampling areas must be used only for the production processes for which they are intended, and they should be isolated from all other areas. Efficiently organized ventilation creates the most favorable sanitary conditions. However, dust trapping right at the place of its formation can be the only fully effective means for controlling dust in work areas. All sorting tables and hide samplingtables must be furnished with local vacuum dust collectors. A. Ye. Shabalin (1957), an engineer, proposed the most efficient device of this type. The top of such a table has numerous holes, and a dust catcher is installed beneath it. The dust catcher is equipped with a hood that prevents large particles from entering the air line. In order that dust would not rise into the upper zone and into the breathing space of workers at the table, lateral vacuum systems are installed on the side opposite the workplace. The air is cleaned of dust and decontaminated prior to being released into the atmosphere.

In order to reduce dust formation, all in-shop transportation and the loading and unloading of raw hides are mechanized to the maximum. Raw hides are unloaded from rail cars and trucks in the quarantine section by conveyers. Electric loading carts that can lift and move a stack without additional handling are extensively used for in-shop transportation of raw hides. Samples are taken for analysis by special automatic or semiautomatic machines.

The dust concentration of work areas can be reduced by periodically flushing shop passageways with water and systematically cleaning them. After work, all trash and spent salt is removed from the areas. Manual cleaning must be replaced by pneumatic cleaning. Raw hide plants and bases must have a centralized vacuum system for this purpose.

Attention should also be turned to personal resources for protecting workers from dust. The RP-K, F-62Sh, and especially the ShB-1 ("Lepestok") respirators are recommended for protection of lungs against dust. The ShB-1 weighs only 10 gm but its filtering surface is equal to 250 cm^2; breathing resistance is 2-4 mm H_2O, and dust trapping effectiveness is 99.9 percent (S. A. Toropov, 1963). Simple gauze dressings cannot be used, since their effectiveness is too low (only 3-5 percent). Workers must be furnished with special hooded overalls offering protection against dust.

Every day after work, special clothing must be vacuum-cleaned in a special area and boots must be carefully washed with hot water before entering the decontamination center.

Disinfection Measures Implemented at Raw Hide Plants and Bases.

Disinfection measures acquire special significance at raw hide plants and bases, since surfaces of production areas processing anthrax-infected raw hides are constantly infected by microflora.

Such enterprises possess their own disinfection unit (the LSD-2m). Thus they can disinfect and whitewash production buildings whenever necessary. Disinfection is performed not less than once every 15 days. The plant's operation is planned in such a way that buildings to be disinfected contain no hides or other raw materials. Quarantine sections, hide sorting and sampling areas, and raw hide disinfection areas are disinfected especially carefully.

One of the following disinfecting agents is used to decontaminate production areas, personal facilities, and plant territory: clarified calcium hypochlorite or hypochlor solution containing 5 percent active chlorine, hot 10 percent caustic soda solution, 4 percent formaldehyde solution (T. A. Trzhetsetskaya, 1957). Prior to disinfection, dust and trash are carefully removed from floors, walls, and equipment. Then one of the recommended disinfectants is applied three times at 1-hour intervals. Walls and ceilings are whitewashed concurrently with disinfection.

Automatic and semiautomatic hide sampling devices are washed with 4 percent formaldehyde solution, or they are wiped with a rag wetted with formalin-turpentine emulsion containing 4 percent formaldehyde solution and 20 percent turpentine solution.

Raw hide stacks and unnecessary articles must not be stored in the plant's yard, since this would hinder insolation and convenient disinfection of the yards, which is done not less than once every 15 days with the same disinfectants used for building disinfection. After every unloading of anthrax-inspected raw hides, the rail bed is carefully disinfected. Unloaded rail cars are vacuum-cleaned, and their outer surfaces are subjected to initial disinfection.

The special clothing and underwear of workers are disinfected once a week, and daily when working with infected raw materials (page 179).

After raw hides and trash and spent salt containers are unloaded from trucks, the latter are manditorily disinfected at the disinfection center with 4 percent formaldehyde solution.

All runoff and waste water on the plant territory is disinfected in a chlorinator. Only after this can it be dumped in the general sewage system.

Disinfection of Raw Hides, Furs, Wool, and Other Raw Materials of Animal Origin

Disinfection of Raw Hides

Hides having a positive precipitation reaction to anthrax are removed from bundles (piles) and stacks and incinerated, while the rest of the hides in the given bundle (pile) or stack are disinfected by pickling in a solution of hydrochloric acid and table salt, or acidified sodium fluosilicate solution, in accordance with instructions approved by the USSR Ministry of Agriculture Main Veterinary Administration.

Raw hides and furs are disinfected in a specially outfitted area insuring isolation of decontaminated raw materials from undecontaminated materials. Wet disinfection entails the use of tanks, vats, paddle wheel tanks, or drums installed in a wall opening between clean and dirty sections.

To keep the disinfectant's temperature at the required level during disinfection, a coil of acidproof tubing is installed at the bottom of the vat or tank. A wooden grate is installed 5 cm above the coil to prevent spoilage of the raw hides due to contact with the heated coil.

Disinfectant preparation containers, drums used to neutralize hydrochloric acid in hides following disinfection, trestles to transport raw hides, and preservation racks are installed in the disease-free section.

Prior to disinfection, the weight of the raw materials and the quantity of disinfectant required by materials preserved by different means, corrected in relation to fresh-dried hides, are determined. Correction factors are used for this purpose.

The disinfectant is first heated to 40-43°. Care is taken to see that the solution temperature would not exceed 39-40° after the dry skins are loaded, and 34-35° after steamed and wet-salted hides are loaded.

Hides are loaded into a tank in such a way that the disinfectant would cover the entire surface of each hide. For this purpose the hides are unfolded so that no folds and bends remain, and they are immersed with the flesh side downward. After the hides are loaded, they are stirred with a wooden paddle, and a grate is installed in the tank to keep them from surfacing.

Presoaking may and may not be performed when disinfecting light fresh-dried and dry-salted hides by pickling, and when disinfecting wet-salted and frozen hides, both light and heavy. Heavy fresh-dried and dry-salted hides are soaked prior to disinfection.

For more reliable hide disinfection, the pickling solution must contain 2 percent hydrochloric acid and 10 percent table salt throughout the entire disinfection process.

Hydrochloric acid is capable of binding with the hide substance itself, and consequently it is removed with the hide from the pickling solution. To replenish this loss and in order to maintain the necessary percent concentration in the tank throughout the disinfection time, the latter is added in excess quantity not exceeding 0.5 percent of the solution quantity, or 5 percent hydrochloric acid in relation to the weight of the hides undergoing disinfection, corrected for hides preserved by fresh-drying.

The quantity of pickling solution employed is equivalent to ten times the weight of fresh-dried raw hides undergoing disinfection.

Water locked within steamed, frozen, wet-salted, and dry-salted hides should be accounted for when preparing pickling solution to be used for hide disinfection.

The pickling solution is prepared as follows: A precalculated quantity of salt is dissolved in two-thirds of the volume of water required. The mixture is carefully stirred until solution sampled from the bottom of the container does not contain salt granules.

Then the appropriate quantity of hydrochloric acid is added, the lacking amount of water is poured in, the solution is heated to the required temperature, and the raw hides are immersed.

The concentration of hydrochloric acid in the solution is checked by titration before and after disinfection.

Frozen raw hides are arranged on wooden poles above the tank before disinfection in such a way that liquid would drain from the thawing hides into the tank. The hides are thawed to 0° in about 12 hours. The poles on which the frozen raw hides were laid are burned or dependably decontaminated.

The hides are kept in the pickling solution for 40 hours at 30°. The temperature is checked every hour with a thermometer lowered into the solution.

After disinfection the paddle wheel tank or drum (tank, vat) is unloaded, and the hides are hung for 2 hours in the clean section of the chamber above a tank to permit drainage of liquid. After this time, the hides are piled in a vat for 1.5-2 hours of neutralization. Neutralization is performed with a solution containing 6 percent table salt, to which 0.5 percent soda ash (in relation to the weight of the raw hides) is added in several batches until neutralization is finished. Each kilogram of hides takes 4 liters of neutralizing solution (a liquid ratio of 1:4), which must have a temperature of 30°. The conclusion of neutralization is established by indicators (an alcohol solution of methyl red or bromcresol purple). Following neutralization, the hides are carefully rinsed with water for 10-15 minutes to remove salts.

Pickling solution that is not too contaminated can be used three times, if prior to disinfection of a new batch of raw hides appropriate quantities of hydrochloric acid and table salt are added to it, such that the concentrations of the ingredients would correspond to the requirements imposed on pickling solution freshly prepared for disinfection. Previously used pickling solution must be replenished such that prior to repeat disinfection its volume would be equivalent to 10 times the weight of the new batch of hides (converted to fresh-dried raw hides). The strength (concentration) of

spent pickling solution is established in the laboratory by titration. Highly contaminated pickling solution is no longer useable.

When heavy fresh-dried and dry-salted raw hides are to be disinfected by pickling following presoaking, the hides are mandatorily soaked in a solution containing 0.05 percent hydrochloric acid and 5 percent table salt.

Each kilogram of fresh-dried raw hides takes 5 liters of this solution. The weight of dry-salted raw hides is converted to dry weight. Hides must remain in the soaking liquid not less than 48 hours at 30°. During this time the hides are carefully stirred every 6 hours (from the loading side of the chamber).

After soaking is finished, a solution of hydrochloric acid and table salt equivalent in volume to that of the soaking water is prepared in a special vat located in the unloading side of the chamber. The solution must contain 5 percent hydrochloric acid. Table salt is added depending on its concentration in the soaking water. Because the hides are soaked in a solution containing 5 percent table salt, 15 percent salt is added to the hydrochloric acid solution. The prepared solution is heated to 40-43°. After the soaking time lapses, the prepared heated solution of hydrochloric acid (5 percent) and salt (15) is poured into the vat containing the hides while continuously stirring. The solution volume must equal the volume of soaking water employed. Thus after this solution is added to the vat, the concentration of hydrochloric acid and table salt would be that necessary for disinfection by pickling.

Disinfection time is said to begin at the moment addition of the solution of hydrochloric acid and salt to the soaking water ends. The entire disinfection process takes 40 hours. The solution temperature must be 30°, and the initial concentration of hydrochloric acid in the solution must be 2.5 percent. After disinfection, the hides are subjected to neutralization.

Hides superficially infected with anthrax microbe spores are disinfected with an acidified solution of sodium fluosilicate. This pertains to both heavy hides (from cattle and horses) and light hides (from small horned animals) preserved by various methods. The solution must contain: 1 percent sodium fluosilicate, 0.7 percent sulfuric acid corrected for 100 percent H_2SO_4, and 10 percent table salt. Every kilogram of fresh-dried hides takes 10 liters of solution (the liquid ratio is 1:10).

The disinfectant is prepared in thoroughly washed tanks, vats, and drums made from wood or some other acidproof material as follows: Sodium fluosilicate is added to a measured quantity of water heated to 40-42° at a rate of 10 gm per liter. The solution is carefully mixed, sulfuric acid is added at a rate of 7 gm per liter (corrected for 100 percent H_2SO_4), the solution is once again stirred, and then table salt is added at a rate of 100 gm per liter. The liquid is once again stirred until the sodium fluosilicate is completely dissolved. The solution is prepared with technical-grade sodium fluosilicate containing not less than 95 percent Na_2SiF_6; technical-trade sulfuric acid containing not less than 75 percent chemically pure acid; technically pure white table salt not containing organic contaminants, iron, and acid-binding substances.

Hides to be decontaminated are immersed in prepared disinfectant, and kept there for 48 hours at 35°. During disinfection, the skins are carefully stirred every 3 hours during the first day and every 6 hours during the second day. Following disinfection,

the hides are neutralized, and salts are rinsed off. Disinfection of raw hides with acidified sodium fluosilicate solution replaces soaking in plant conditions. Following disinfection, the hides are forwarded for plant processing or for salt preservation.

Disinfection of Raw Furs

Raw furs superficially contaminated or suspected of being contaminated by anthrax pathogen spores, both fresh-dried and wet-salted, are disinfected in a solution of hydrochloric acid and table salt following presoaking in acid cereal drench. Raw furs preserved by fermentation are decontaminated without presoaking.

Acid cereal drench of the following composition is prepared for soaking prior to disinfection of fresh-dried and dry-salted raw furs in a drum (tank, vat): 12 liters of water for every kilogram of dry furs (liquid ratio 1:12), 90 gm oatmeal per liter of water, and 30 gm table salt per liter of water. The prepared liquid is allowed to ferment for 25 hours, during which time the temperature is kept at 35°.

Then the acidity of the liquid is determined. Corrected for acetic acid, it should be 2-4 gm per liter. If acidity is below 2 gm per liter, the fermentation time is lengthened until the required acidity is attained.

After this the raw furs are loaded into the drum (tank, vat) for 3 days of soaking. The drum is revolved two or three times during loading, and subsequently it is revolved every 8 hours for a period of 5 minutes (the contents of tanks and vats are stirred correspondingly). After the soaking time lapses, 2 percent hydrochloric acid (corrected for HCl) and 7 percent table salt are added to the same liquid in the drum without taking the furs out. This would increase the concentration of table salt in the liquid to 10 percent. The hydrochloric acid and table salt are initially dissolved in a fourfold quantity of soaking liquid taken from the same drum (tank, vat). After the solution of hydrochloric acid and table salt is added, the drum is turned for 10 minutes; subsequently it is turned every 8 hours for 5 minutes. Disinfection time is 40 hours at 30°.

Following disinfection, the raw furs are rung out in a centrifuge and neutralized in a different drum or vat. A solution having the following ingredients is used for neutralization: 6 parts water to 1 part centrifuged raw furs, 5 gm soda ash per liter of water, and 50 gm table salt per liter of water.

The temperature of neutralizing solution prior to loading must be 30°, and neutralization time is 1.5 hours. After loading, the drum is turned for 5 minutes, and then it is turned every 30 minutes for 5 minutes. After neutralization, the raw furs are rung out and immediately forwarded to the fur processing plant for further processing.

Solution intended for drum disinfection of raw furs preserved by fermentation is prepared such that there would be 10 liters of liquid containing 2 percent hydrochloric acid (corrected for HCl) and 10 percent table salt per kilogram of raw furs. The solution temperature prior to loading of the raw material must be 35°.

After the raw material is loaded, the drum is turned two or three times, and subsequently the drum is turned for 2-3 minutes every 6 hours. Disinfection time is 40

hours, and solution temperature is 30°. Following disinfection, the raw material is centrifuged and neutralized, after which it is once again centrifuged and forwarded to the fur processing plant for further processing.

Tanned sheepskins are disinfected in formalin vapor chambers at 62-63°; formalin consumption is 250 ml per cubic meter of chamber volume, and exposure time is 2 hours and 30 minutes. Sheepskins are placed in the chamber in such a way that they would not touch one another. The chamber's steam inlet is covered with burlap to prevent spoilage of the sheepskins.

A. A. Polyakov, V. I. Ivanova, and A. A. Shakhalin (1968) suggested a solution for simultaneous disinfection and preservation of raw hides (sheepskins, goatskins, calfskins) superficially infected by anthrax pathogen spores. The solution contains 2 gm nicotine iodochloride chlorhydrate ("niran"), 5 gm potash alum, and 3 gm sodium fluosilicate per liter, and 26 percent table salt. Exposure time is 30 hours, during which the raw material is stirred periodically. After being processed by this solution, the raw hides need not be neutralized, rinsed, and preserved, as a result of which the labor outlays are reduced and the hide processing time is decreased.

"Cold sterilization" by gamma-rays produced by decay of the radioactive isotope cobalt-60 is presently the most promising method for decontaminating raw furs, hides, and wool.

Research by V. V. Arkhipov (1959), V. I. Ryakhovskiy (1961), V. V. Arkhipov, M. S. Lyuksemburg, and G. S. Agafonova (1963), V. D. Bubnov (1969), and others showed that when raw hides, fur, wool, down, and feathers are irradiated at a dose of 1.8-2.5 mrads, any anthrax pathogen spores present die. The density of high infestation and the species of animal from which the hides were obtained do not significantly influence the size of sterilizing gamma-ray doses. High-power gamma-ray units permit decontamination of raw hides and wool without unbaling. Installation of such gamma-ray sterilizers at major ports will make it possible to decontaminate imported raw materials of animal origin, and thus insure receipt of sanitary raw materials by industrial enterprises.

A. A. Polyakov, G. D. Volkovskiy, and T. A. Trzhetsetskaya (1974) proposed OKEBM gas (a mixture of ethylene oxide and methylbromide at a weight ratio of 1:2.5) for decontamination of fresh-dried raw hides superficially infested by anthrax spores. This gas has high sporicidal action and penetrability, it causes absolutely no spoilage of the material being processed, it volatizes readily with ordinary airing, and it can decontaminate packaged materials in chambers or beneath tents made from brand PK-4 polyamide film.

Polyamide film may be glued with special brand PK-4 glue, which permits manufacture of tents of any needed size. Fresh-dried raw material with a moisture content from 6 to 18 percent is stacked with the packages spaced 25-30 cm apart on a specially allocated dirt platform and covered with polyamide film. The tent is sealed off by covering the lower edges of the film with dirt or by immersing them in water. Liquid gas is fed beneath the film from a tank through special nozzles and a rubber hose; the gas is distributed through a T-branch to containers installed in the upper horizon of the tent.

In this closed system the prescribed dose of the liquid gas evaporates slowly, and since the gas phase is characterized by a high vapor pressure, it penetrates into the material undergoing decontamination, where it is gradually absorbed. Disinfection occurs at a certain gas concentration, exposure time, temperature, and humidity. Fresh-dried raw hides are decontaminated at a temperature not lower than 10°. The gas consumption rate per cubic meter of enclosed volume is 3 kg with an exposure time of 10 days, 2 kg with a 15 day exposure time, or 4 kg with a 7 day exposure time.

After the prescribed exposure time lapses and the material is decontaminated, the polyamide film is removed, and the hides are aired to allow escape of the gas.

The tightness of the tent is monitored during decontamination on the basis of change in the color of the flame of a haloid indicator burner.

Safety rules must be complied with strictly during disinfection. An open flame must not be used near the gas tanks. The ingredients of OKEBM gas are toxic to man when inhaled, and they cause burns on coming in contact with skin; this is why all work with OKEBM is done in gas masks outfitted with a brand "A" (brown) filter box. Personnel cannot remain in the gas-filled zone for more than 30 minutes, and they cannot remove their gas masks for 2-3 minutes after leaving the gas-filled zone. In addition to a gas mask and gloves, disinfection specialists wear overalls, rubber boots, and aprons. Warning signs are posted not less than 50 meters from the disinfection site: "No Entry" and "Danger, Poisonous Gas". The place of gas decontamination is patrolled around the clock. The guard must remain on the windward side and not less than 20 meters from the decontamination site. After work, special clothing and footwear is aired for not less than 4 hours, and it is stored in an area not frequented by personnel.

Disinfection of Wool and Goat Hair

Wool and goat hair infected with anthrax is disinfected in Krupin chambers with live steam at a pressure of 0.5 atmospheres (temperature 111-112°).

Bags containing loosely packed wool must not touch one another inside the chamber. The chamber is loaded with wool at a rate of not more than 50 kg per cubic meter of chamber volume. Disinfection time is 1 hour 45 minutes for wool-filled bags weighing 50 kg, 1 hour 20 minutes for a weight of 30 kg, and 1 hour 10 minutes for wool-filled bags weighing 20 kg.

Goat hair is disinfected in bales weighing not more than 30 kg. Exposure time is 1 hour 30 minutes. The loading limit of a disinfection chamber must not exceed 50 kg of goat hair per cubic meter of chamber volume.

Cold-washed wool can be disinfected in formalin vapor chambers. The wool is spread on thin rope screens as a layer not more than 5 cm thick, at a rate of 3 kg per cubic meter of chamber volume. Then the temperature in the chamber is raised first with a heater to 40° for 30 minutes, and then with live steam to 50-55°, at which time formalin is sprayed in at a rate of 160 ml per cubic meter of useful chamber capacity. Disinfection continues at 62-65° for 1 hour 30 minutes, after which the chamber is aired out and the raw material is unloaded. Disinfection specialists must work in gas masks.

A small quantity of wool can be disinfected by wetting it in 2.5 percent formaldehyde solution for 10 hours at room temperature.

The work area used for wool disinfection is divided strictly into clean and dirty sections. A vat, tank, or barrel is placed in an opening in the wall separating these sections. One half of the container protrudes into the loading section, and the other half protrudes into the unloading section (the clean section). There is a separate lid for each half of the container. Hot (40-45°) formaldehyde solution is poured into the tank at a rate of 6 liters per kilogram of wool. The wool is immersed in small batches so that it is entirely covered with solution. After disinfection, the wool is removed and spread on a raised trough-shaped platform installed in such a way as to permit liquid draining from the wool to return to the tank. Then the wool is dried. The solution is used up to five times. With each use, the solution is titrated and consumed formaldehyde is replenished.

All recommended disinfection methods are intended for decontamination of a small quantity of wool. Moreover special equipment is required, the methods are laborious and hazardous to workers, and they cause significant deterioration of wool quality.

In 1973 A. A. Polyakov and G. D. Volkovskiy suggested decontaminating baled wool with OKEBM gas.

Wool to be disinfected is transported, with the necessary precautions, to a prepared awning-covered platform, or it is laid in areas that must be not less than 50 meters from residential and production buildings.

The bales (bags) of wool are placed 25-30 cm apart in several rows and tiers. When bales are arranged in several tiers, there must be a 30 cm air gap between tiers to permit circulation of the gas-air mixture. Wool loading density must not exceed 120 kg per cubic meter of tent volume. A light framework extending 50 cm above the top tier of wool is assembled over the wool. Containers for liquid OKEBM mixture are suspended every 10 meters from the upper rack of the framework. A ditch 40 cm deep is dug 15-20 cm from the edge of the framework. A film tent is carefully stretched over the framework, and its ends are lowered into the ditch, which is then filled with dirt and packed down well. Then liquid OKEBM mixture is fed from a tank through special pipes into the containers installed beneath the film.

Given a temperature of 15° and higher beneath the film tent and a loading density of not more than 120 kg of wool per cubic meter of enclosed volume, wool in bales (bags) is decontaminated with OKEBM mixture consumed at a rate of 4 kg per cubic meter in 10 days, and 3 kg per cubic meter in 15 days. After the exposure time lapses, the tent is removed and the wool is aired. The windows and doors of the building are opened and fans are turned on for this purpose. When the air temperature is 10-20°, it takes 10 days to completely air the wool, while when the temperature is above 20°, airing is completed in 7 days. Then the wool is left out in the open beneath an awning until complete disappearance of the characteristic odor.

Disinfection of Bristle and Hair

Raw bristle and hair infected with anthrax are disinfected in Krupin chambers with flowing steam in the same way as wool. Exposure time for bristle packed in 15-30 kg bags is 1 hour. The exposure time for bags containing hair weighing up to 30 kg is

1 hour 10 minutes, and that for bags weighing from 30 to 50 kg is 1 hour 30 minutes. A chamber can be loaded with raw bristle and hair at a rate of up to 100 kg per cubic meter. Semifinished bristle and hair products packed in boxes are disinfected by drilling holes through the latter and through wrapping paper.

Boxes are separated by wooden beams in the chamber. Boxes containing hair are kept in the chamber for 2 hours, while boxes of bristle are kept there for 3 hours.

Disinfection of Bone

Bone collected in the field and bone of unknown origin is disinfected in covered tanks. The tank is set up such that half of it is in the loading section and the other is in the clean section. Disinfection is performed with a calcium hypochlorite solution containing 5 percent active chlorine, the exposure time being 6 hours. When 4 percent formaldehyde solution is used, the exposure time is 2 hours. Bone is immersed into the disinfectant at a rate of 4 liters of solution per kilogram of bone, such that there would be at least a 10 cm layer of the solution above the bone. Solution temperature must not be below 15°. Following disinfection, the bone is dried (T. A. Trzhetsetskaya).

Preventive Measures Against Anthrax During Preparation of Reservoir Beds

Anthrax pathogen is known to be capable of surviving for a rather long period of time in soil, and spores may even germinate. At a certain moisture content (up to 80 percent) and temperature (above 12°) anthrax pathogen spores germinate in chernozem and humic soil, and the number of colonies produced by inoculations made with this soil increases. Anthrax spores have been discovered at animal burial pits in humic or peaty ground 30 years after burial of the last anthrax-stricken carcass (N. A. Pokshishevskiy, A. D. Golovin, 1931; A. A. Vladimirov, I. A. Baytin, 1940).

Analyzing 13 soil samples from the yards of raw hide warehouses, A. O. Ozol and N. I. Vinogradov (1929) discovered anthrax pathogen in three samples.

According to V. V. Arkhipov (1957) an animal burial pit over which a lake formed following a major flood served as source of infection causing an anthrax epizootic. After 20 years, half of the lake dried out (in relation to surface area), and animals began to be tended on land relinquished by the water. In a few days an anthrax epizootic broke out.

Z. A. Yelistratova (1959) studied samples of dirt from a 40-year-old animal burial pit. She isolated nine strains that were identical in morphological, hemolytic, and virulent properties to anthrax pathogen strains.

Similar results are also reported by B. P. Novikov (1960), Yu. I. Boykov (1964), I. N. Presnov (1966) and other authors.

Anthrax agents may also be detected on the sites of former primitive tanneries and other enterprises involved in the initial processing of raw materials of animal origin, since the soil of these places is usually rich in organic matter and serves as a favorable medium for development of *Bac. anthracis*.

During periods of high water and intense rains, spores may be carried great distances by water, settling on flooded meadows and pastures. Animals grazing in such places often pull out and swallow grass together with roots, which may bear dirt mixed with anthrax pathogen. Drinking from infected water basins, animals muddy the water and swallow anthrax spores together with the mud.

That the pathogen can survive for a long period of time in soil must be accounted for when building canals and reservoirs. Thus when planning and implementing anti-anthrax measures, special attention should be turned to disinfecting animal burial pits, especially ones located within the construction zone of a hydroelectric power plant and a reservoir bed. Construction operations associated with preparation of a reservoir bed and proceeding in places infected by anthrax require implementation of a broad complex of veterinary-sanitary measures. Prior to allocation of land areas for exploratory, construction, and other operations involving removal or movement of dirt, zones of flooding and the routes of canals, irrigation systems, and water supply systems must be subjected to epizootiological analysis, which requires selection and analysis of reference data on the sanitary condition and the anthrax-related morbidity and mortality dynamics of agricultural animals. The locations of animal burial pits, places of unsanctioned animal burial, biothermic pits, animal farms at which cases of animal deaths due to anthrax had been observed, to include yards belonging to kolkhoz farmers, laborers, and white collar workers, the sites of primitive tanneries and facilities utilizing animal products ("tallow-melters"), and enterprises of raw hide industry must be revealed precisely in this case.

The veterinary and medical services determine the possibility for construction depending on the analysis results, and they indicate the sort of sanitary and preventive measures that must be implemented at the time of construction operations, at the stage of the architectural planning assignment. Permission to allocate land for operations associated with removal or movement of dirt is granted on the basis of the conclusions of a commission in which representatives of the veterinary and epidemiological services manditorily participate.

Wooden structures, floors, wooden foundations, manure, and trash of little value at farms and enterprises harboring anthrax pathogen are burned on the spot. Soil beneath buildings, in cattle pens, and in fattening yards is irrigated with decontaminants. Then the first 25 cm of soil is carried outside the zone of sanitary cleansing to a special area where it is decontaminated with OKEBM gas beneath PK-4 polyamide film. After this dirt is removed and carried away, the territory of infected farms is covered with dry calcium hypochlorite at a rate of 5 kg per square meter. Then the land is moistened and plowed to a depth of 25 cm.

Liquid manure is decontaminated with calcium hypochlorite in liquid manure collectors (page 179). Biothermic pits and liquid manure collectors are covered with a layer of dry calcium hypochlorite not less than 5 cm thick. The latter is covered with clean dirt and a layer of clay 50-70 cm thick, after which a layer of concrete not less than 50 cm thick is laid.

All burial places in which animals killed by anthrax had been buried and which are within the zone of flooding, the zone of rising ground water, in areas of shore erosion, or at sites of ditch digging are moved away or decontaminated.

In some cases, depending on the nature of wave action, the type of bedding rock, and the topographic conditions, dirt within the limits of an animal burial pit or the location of a single buried carcass can be stabilized, on the condition that such stabilization guarantees against erosion.

Movement of animal burial pits or individual buried carcasses of animals killed by anthrax is permitted, if the following veterinary-sanitary rules are complied with.

Permission to reserve a land area to which to move an animal burial pit is granted in the established order on the consent of veterinary and epidemiological inspection organs. In order to protect animals from anthrax within a radius of up to 15 km around the zone of operations associated with removal and transportation of dirt, all animals susceptible to anthrax are subjected to preventive immunization. Agricultural animals cannot be grazed in areas of earth-moving operations or near the route used to transport animal remains to be reburied, and dirt. The zone of earth-moving operations in the vicinity of the animal burial ground being moved and the new burial pit, the route used to transport animal remains and dirt, and parking areas for equipment used in the earth-moving operations are declared to be a prohibited zone for people and transportation not directly involved in the operations, and for animals. The hazardous zone is fenced off, and warning signs are posted: "Anthrax, No Trespassing".

Residential buildings and the food services units or eating places for workers in the zone are located 1,000 meters away from earth-moving operations (with a consideration for the wind rose). All workers may enter and leave the hazardous zone only through a decontamination center. An OPPK is used to disinfect special clothing and special footwear.

Persons directly involved in movement of anthrax burial pits must be immunized against anthrax not less than a month before starting work, they must be briefed on personal preventive measures before work, they must live in specially allocated quarters, and they must remain under constant medical observation throughout the entire period of work, and for 10 days after it is finished. Persons involved in the reburial of dirt and animal remains must be furnished with sanitary protective clothing (rubber boots, rubber oversleeves, overalls, headwear, and a respirator covering the mouth and nose).

Animal remains and dirt are reburied in special trenches not less than 2 meters deep. The trenches are dug in such a way that vehicles carrying infected dirt would drive up from one side while clean dirt removed from the trench, with which the latter is to be covered, would be on the other side of the trench.

All work must be maximally mechanized, and the bodies of dump trucks are sealed off and the sides are reinforced to prevent spilling of dirt at the time of its shipment.

Dirt is removed from the territory of the animal burial pit by an excavator to a depth of 3 meters. Dirt and, as they are removed, animal remains are wetted (to prevent dispersal) with 20 percent calcium hypochlorite solution containing not less than 5 percent active chlorine, and loaded into dump trucks while wet. The contents are covered with a tarpaulin, also wetted with calcium hypochlorite solution, or with polyethylene film.

After the dirt is removed, a 2 meter layer of uncontaminated soil is dumped into the new pit, the soil is packed down, a fence is put up 50 meters from the pit, and a trench is dug along its perimeter.

Tools, vehicles, and excavators do not leave the limits of the animal burial pit and they are not used for other purposes until work is finished. During the work, the equipment and tools are subjected to regular disinfection in a specially allocated area.

Disinfection is performed with one of the following disinfecting agents: 4 percent chloramine or preparation KhB solution; 5 percent formaldehyde solution mixed with 5 percent soap solution heated to 60°; 6 percent formaldehyde solution mixed with 0.5 percent detergent heated to 50-60°; 6 percent hydrogen peroxide solution mixed with 0.5 percent detergent.

After work is finished, the equipment and tools are subjected to final disinfection by two copious applications of one of the disinfectants listed above. The driver's cab is disinfected as well (seats and rubber mats are disinfected in an OPPK). Another method is to first fire all iron parts with a VDM blowtorch, after which they are flushed with a beating stream of formalin-kerosene emulsion consisting of 10 parts 40 percent formalin, 10 parts kerosene, 6 parts Creolin, and 75 parts water, heated to 65-70°.

Disinfection of Anthrax Animal Burial Pits

There were no effective methods for sanitizing animal burial pits until recently. The existing chemical and thermal methods of soil decontamination could be used only in certain cases, in small areas of insignificant depth. Biological methods of burial pit decontamination--creating particular ecosystems in the soil, planting vegetation, or introducing microbes antagonistic to anthrax pathogen into the soil--require further study.

Though sanctioned by the manuals, the practice of laying concrete over anthrax burial pits within the "stagnant" zone of a future reservoir is no guarantee against possible escape of the infectious agent through pores in the concrete. Even with the best organization and with maximum mechanization of the operations, movement of anthrax burial pits is laborious, expensive, hazardous to workers, and, what is most important, it does not destroy the anthrax pathogen.

A. A. Polyakov and G. D. Volkovskiy (1968-1974) developed a method for disinfecting anthrax-contaminated soil covering large areas (2,000-3,000 m^2) with OKEBM gas beneath synthetic polymer film. The main advantage of this method is that it kills the infectious agent in a soil layer up to 200 cm deep right where it lies. It does not require the use of digging equipment, it is technically simple, and it does not create an epizootic hazard. The cost of the method is three to four times lower than that of moving an animal burial pit.

The method basically involves covering the area to be decontaminated with polyamide film, the margins of which are sealed off by being covered with dirt or water. Then a prescribed dose of liquid OKEBM gas is fed into previously distributed containers. The liquid OKEBM gas is dispensed by weight, observing the safety rules of working with tanks containing liquefied toxic gas. Personnel work in gas masks, rubberized aprons, and rubber boots and oversleeves.

Slowly evaporating gas fills the air space above the soil, which gradually absorbs it, causing its decontamination.

Sandy loam soil is decontaminated to a depth of 2 meters with OKEBM gas beneath a film at a soil temperature not less than 10° in two variants. In the first, holes 100-200 cm deep and not less than 15-20 cm in diameter are dug in the area to be decontaminated, one hole for every 20-25 m^2. The gas is dispensed at a rate of 3 kg per square meter of soil, and the gas containers are located 20 meters apart. Exposure time is 20 days. Holes are not dug in the second variant, which requires a gas dose of 5 kg per square meter of contaminated area and an exposure time of not less than 45 days.

After the required exposure time lapses, the film is removed and the soil is aired to permit the gas to escape. Safety measures (page 202) must be complied with strictly when disinfecting soil. Soils of other types can also be decontaminated with OKEBM gas. It should be kept in mind, however, that if there are large numbers of vertical passageways in the soil--earthworm migration paths--the gas may descend to considerable depth along these passageways too quickly to permit its absorption by the soil in adequate quantities, and the latter's decontamination. Therefore before decontaminating such soils, the ground must be dug up to the greatest depth possible in order to break up the vertical passageways.

BIBLIOGRAPHY

1. Arkhipov, V. V., Lyuksemburg, M. S., and Agafonova, G. S., TRUDY VNIIVS, No 22, 1963, p 76.

2. Boykov, Yu. I., TRUDY VNIIVS, No 24, 1964, pp 107-125.

3. Boykov, Yu. I., "Problemy veterinarnoy sanitarii" [Problems in Veterinary Medicine], Moscow, Izd-vo "Kolos", 1964, p 56.

4. Boykov, Yu. I., TRUDY VNIIVS, No 25, 1966, p 85.

5. Boykov, Yu. I., TRUDY VNIIVS, No 29, 1967, p 23.

6. "Veterinarnoye zakonodatel'stvo" [Veterinary Legislation], Moscow, Izd-vo "Kolos", Vol 1, 1972, p 151.

7. "Veterinarnoye zakonodatel'stvo," Moscow, Izd-vo "Kolos", Vol 2, 1972, pp 311, 319, 336, 341, 399, 439, 480, 485, 488, 518.

8. Vladimirov, A. A., and Baytin, I. A., "Sibirskaya yazva" [Anthrax], Moscow, Sel'khozgiz, 1940.

9. Vyshelesskiy, S. N., and Terent'yev, F. A., "Chastnaya epizootologiya" [Specific Epizootiology], Moscow, Sel'khozgiz, 1954, p 10.

10. Volkovskiy, G. D., "Voprosy effektivnosti protivosibireyazvennykh meropriyatiy" [Questions of the Effectiveness of Anthrax Control Measures], Moscow, 1974, p 173.

11. Volkovskiy, G. D., and Trzhetsetskaya, T. A., "Voprosy effektivnosti protivosibireyazvennykh meropriyatiy," Moscow, 1974, p 186.

12. Yelistratova, Z. A., TRUDY TSNIDI, No 11, 1959, p 88.

13. Polyakov, A. A., "Veterinarnaya dezinfektsiya" [Veterinary Disinfection], Moscow, Izd-vo "Kolos", 1964.

14. Polyakov, A. A., "Osnovy veterinarnoy sanitarii" [The Fundamental Principles of Veterinary Medicine], Moscow, Izd-vo "Kolos", 1969.

15. Polyakov, A. A., and Volkovskiy, G. D., "Voprosy effektivnosti protivosibireyazvennykh meropriyatiy," Moscow, 1974, pp 145-152.

16. Peskov, P. G., "Veterinarno-sanitarnyy nadzor na zheleznodorozhnom transporte" [Veterinary-Sanitary Inspection of Rail Transportation], Moscow, Izd-vo "Kolos", 1969.

17. Ustenko, V. S., TRUDY VNIIVS, No 24, 1964, pp 94-101.

18. Ustenko, V. S., TRUDY VNIIVS, No 29, 1967, p 428.

19. Kharisov, Sh. Kh., Abdullin, Kh. Kh., and Ryzhov, L. I., VETERINARIYA, No 6, 1969, p 99.

20. Yarnykh, V. S., "Mekhanizatsiya veterinarno-sanitarnykh rabot" [Mechanization of Veterinary-Sanitary Operations], Moscow, Izd-vo "Kolos", 1965.

21. Lefevre, D., "Contribution a l'etude du charbon professionnel on France," Lille, 1939.

22. Materna, A., ZBL. BAKT., No 48, 1930, p 11.

23. Maugeri, S., ARCH. HYG., No 111, 1934, p 5.

24. Seidel, G., "Zschr. fur Gesichte der Naturwissenschaften, Technik und Med.," 1960, pp 72-92.

25. Seidel, G., "Zschr. fur gesamte Hyg. und ihre Grenzgebiete," Vol 9, 1963, pp 688-700.

26. Zettl, K., and Kauker, E., BERL. MUNCH. TIERARZTL. WSCHR., No 72, 1959, p 21.

BIOLOGICAL SOIL DECONTAMINATION

The biological method is extensively employed today to control insect pests, to protect agricultural crops against diseases, and to control fruit pests. We recommend this control method for decontamination of anthrax-infected soil as well.

The fact that soil serves as a reservoir for the causative agents of several infectious animal diseases has been known for a long time. Even in ancient times, people distinguished "healthy" soil from "unhealthy" soil. They classified upland, dry, sunlit soil as "healthy" as a rule and lowland, marshy soil as "unhealthy".

In 1877 Pasteur and Zuber published a report that *Bacillus pyocyaneus* settling from the air suppressed growth of anthrax microbes in nutrient medium. This report attracted the interest of scientists.

Interest toward antagonist microbes grew even more after antimicrobial substances were obtained. The first such preparation was penicillin, obtained in 1941; the second--streptomycin--was obtained 3 years later. After this, many researchers began studying the biology of pathogenic microorganisms inhabiting the soil.

Attempts at finding chemical compounds with which pathogenic microbes could be destroyed in soil were undertaken beginning at the end of the 19th century. Scientists turned their greatest attention to soil from which they were able to isolate anthrax pathogen. In those years and for a very long time after, solutions of mercuric chloride, calcium hypochlorite, sulfuric and carbolic acids, chlorine gas, chloropicrin, and other chemicals were recommended for decontamination of soil containing *Bac. anthracis*. However, the solutions of many chemicals and gaseous substances were not found to be very effective.

Inasmuch as *Bac. anthracis* may survive for a long period of time in soil, and since it is known from published data that certain constant ecosystems (associations, communities) exist in nature untouched by man, scientists became curious as to the existence of plants that may interact with anthrax microbes in soil in the course of their vital activities, and as to the sort of mutual relationships that might exist. Plants apply selection pressure upon microbes and other organisms about them. Doubtlessly the microorganisms also influence growth and development of the plants.

V. V. Dokuchayev, a Russian scientist who founded soil science, indicated back in 1883 in his doctorate dissertation, "Russian Chernozem" that "soil is a self-contained natural body which, as with any plant or animal organism, lives and changes perpetually, developing at times and breaking down at others, progressing at times and

regressing at others." Soil is a perpetual site of symbiosis, antagonism, and parasitism between countless organisms, including microorganisms, living within it. Throughout all development, plants and microorganisms have been in constant and close interaction. The dynamics of the microflora-plant ecosystem are mutually related with the cycling of matter in nature and with soil-forming processes.

A. A. Isakova (1940) demonstrated that with the first stirrings of life, contacting soil microflora with their roots, plants immediately begin to select microorganisms, which in turn adapt to the former in view of the changes occurring in the environment. This leads to fundamental alteration of the soil microflora. She established that owing to a large, deeply penetrating root system, in 3 or 4 and sometimes 7 years perennial plants can stabilize the changes they effected in the soil microflora.

Yu. M. Voznyakovskaya, A. A. Isakova, et al., and A. I. Korenyako, N. A. Krasil'nikov, et al. (1948-1969) showed that the roots of different plants produce secretions of differing composition and quantity, and therefore the composition of microflora about them varies as well. The authors established that the root secretions of wheat, corn, and flax inhibit or completely halt the growth and development of nitrogen-fixing bacteria as well as rhizomous, cabbage, and potato bacilli, while the root secretions of clover, alfalfa, and peas promote their development.

Beginning in 1930 B. P. Tokin studied phytoncides--protective substances formed by plants. He demonstrated that "plants kill various microorganisms located some distance away...." Eucalyptus, poplar, and birch leaves and pine needles "kill diphtheria pathogen from some distance away....". At the time of their germination, rye, wheat, and barley seeds are not as a rule subjected to the action of bacteria, the numbers of which are in the billions. Cereal plants discharge liquid and volatile bactericidal substances as they germinate.

B. P. Tokin believes that in the course of evolution, every species of plants adapted itself to particular pathogenic bacteria, viruses, fungi, protozoans, annelids, and insects. There are no organisms living in isolation on the globe, the author asserts. Communities (ecosystems) created through evolution exist everywhere--in the forests, the steppes, and the oceans. Owing to discharge of phytoncides into the atmosphere and soil, plants can inhibit or stimulate the growth and development of other plants and their ecosystems.

N. A. Krasil'nikov (1969) suggests that man can alter the composition of soil microflora in any desired direction. Selecting more-active plants and introducing them into crop rotation, in his opinion we can go a long way to eliminate many undesirable species of microbes.

We were unable to find works studying interaction between particular species of plants and anthrax microbes. But some researchers have published extremely valuable statements concerning the use of plants in anthrax control.

Fizhan, a domestic veterinary researcher, concluded in 1893 on the basis of many years of anthrax control in St. Petersburg and other provinces, that in addition to draining marshes, covering the drained areas with cultivated plants must be foreseen among the anthrax control measures. He stated: "...it should be presumed that anthrax bacteria should die in cultivated and exploited soil."

Following a lengthy experiment in anthrax control (1882-1883), A. P. Levitskiy concluded that wherever marshes were drained for sanitary purposes (for example for the purposes of eradicating malignant fevers in man or anthrax in animals), a violent outbreak of disease almost always occurred in the first and sometimes in the second year after draining, after which the disease gradually abated and disappeared.

Guinter (1904) believed that anthrax bacillus is an accidental parasite. It grows fabulously and forms spores in moist areas, and it is consumed accidentally with grass by grazing animals, which then fall ill.

Basing ourselves on the work of N. A. Krasil'nikov, B. P. Tokin, Ye. N. Mishustin, and other researchers, in 1947-1949 we performed experiments aimed at studying the influence of different plants on pathogenic anthrax microbes in soil. In the initial laboratory experiments, plants were grown in bowls and flasks containing soil infected by different methods. It was established that plants influence virulent anthrax microbes in soil in different ways. Lethal action is typical of: clover, rhubarb, and alfalfa--in the second year of vegetation; vetch, winter wheat, spring wheat, rye, and garlic--in the first year of vegetation; onion, hemp--in the period of seed germination. Plants such as potatoes, radishes, and turnips promote proliferation of anthrax microbes and their penetration into underlying soil layers. Under the same conditions, seradella, dakota vetch, oats, barley, buckwheat, millet, lupine, poppy, flax, carrots, radish, rape, watercress, morning glory, and petunias do not have an influence on anthrax microbes in soil.

Anthrax microbes were detected on the leaves and stems of clover, dakota vetch, winter wheat, carrots, and petunias only in the sprouting period, and on wheat-grass, turnips, hemp, and garlic in the first 1-4 months of vegetation. Anthrax microbes carried out of spore-infested soil by garlic leaves were asporogenic in all cases.

Published data and the positive results of our laboratory research permitted us to initiate field experiments (1950-1955). We studied the soil of animal burial pits in which the carcasses of animals killed by anthrax had been buried in different years. One experiment was performed on an animal burial pit with soil exhibiting the highest infestation by anthrax microbes.

The burial pit, which had an area of 950 m^2, was divided into 96 plots after we studied the grass composition and subjected the soil to general analysis. The plots, the plants sown in them, and the bacteriological soil studies were diagrammed. Three plots were allocated to every plant species under investigation. One was experimental and two were controls. Grass from two plots--one experimental and one control--was mowed and burned; the third plot was left in its natural state. The first two plots were plowed to a depth of 25-30 cm. The seeds of the experimental plants were sown in the experimental plot, with a consideration for the agronomic norms. The soil was analyzed before, during, and after plant vegetation. The organs of experimental and control plants were studied as well.

In subsequent years the area of the plots was increased to 100 m^2 and more. In the course of 3 years the plots were sown in spring and fall with spring and winter cereal, pulse, and feed crops.

The field experiments established that winter rye, winter wheat, and perennial winter rye completely eradicated anthrax microbes from the infected soil of the animal burial pit. Data differing from the results of the laboratory experiments were obtained concurrently. Thus, for example, dakota vetch did not have a noticeable influence upon anthrax microbes in laboratory experiments, but it did have a lethal effect upon them in the field experiments.

Numerous laboratory experiments established that under certain conditions (moisture content and temperature) anthrax microbes present in different types of soil (meadow, chernozem, podzolic) germinate in vegetative forms that quickly proliferate in the soil, and that under favorable conditions (a decrease in temperature, a reduction of moisture content, and so on) they revert to the spore form. Similar data were obtained in the field experiments performed at anthrax foci.

According to V. A. Kosarev (1956) corn also has a lethal effect on anthrax microbes; in his experiments the lethal effect of corn was greater upon Tsenkovskiy vaccine No 1 microbes than upon No 2 microbes; its action is apparently even weaker upon epizootic anthrax strains.

Our data on the lethal influence of certain plants upon anthrax microbes in soil were confirmed by experiments performed by I. N. Presnov (1966) and B. Kh. Shushayev (1969).

The mechanism of the antagonistic action plants have on anthrax microbes in soil may be described as follows. By their secretions, plants can have a direct lethal influence upon the vital activities and viability of microorganisms. Moreover plants are capable of attracting, into the zone of their rhizosphere, microorganisms specific to the given species of plants. Being antagonistic to anthrax microbes, these microorganisms can suppress their vital activities. In addition to direct or indirect influence of plants upon microbes, we must also consider the significance of the soil itself, the physical and chemical properties of which go a long way to define the direction of microbiological processes and, to an equal degree, the development of particular species of microorganisms.

Ecosystems are also altered by land improvement operations. Apparently, not only are the natural, soil, hydrological, and climatic conditions improved for agricultural crops as a result, but also favorable cycles (water-air, heat, salt) are created in the soil, which increases the concentration of air in soil; exchange between soil and atmospheric air improves; soil temperature rises; microorganisms become active and their numbers increase; mineralization of plant remains accelerates. Operation of all of these factors together leads both to renewal of plants and improvement of their nutrition, and to change in soil structure and change in the conditions enjoyed by soil microflora and, consequently, to germination of anthrax microbe spores and the death of their vegetative forms.

The second biological method of decontamination of soil containing anthrax microbes involves introduction of antagonist microbes into infected soil.

In 1957-1958 N. A. Krasil'nikov and I tested microorganisms isolated from different soils and studied the action of some new antibiotics upon spore and vegetative forms of anthrax microbes in conventional test objects and in soil.

Antagonistic action upon vegetative and spore forms of anthrax microbes was determined by the following commonly accepted methods.

The first method: After 6 days' growth, we removed "blocks" (round agar plates 0.6 cm in diameter) from cultures of particular actinomycete strains and placed them on agar in bacteriological dishes seeded with spore or vegetative forms of anthrax microbes. The dishes were placed in a thermostat for 10 days, and after 1-2 days the zone of clarification--that is, the degree of influence of the actinomycete or antibiotic upon anthrax microbes--was determined.

The second method: Culture fluid containing a particular actinomycete strain was applied to particular places in bacteriological dishes seeded with spore or vegetative forms of anthrax microbes. After this the dishes were placed in a thermostat, and the zone of clarification was determined in the same way.

The third method: Actinomycete strains or antibiotic preparations which were revealed by the previous methods to have antibiotic action against anthrax microbes were tested for their activity by the serial dilution method. The actinomycete strain or antibiotic under investigation was introduced into a series of test tubes containing different dilutions (1:3, 1:9, and so on) of sterile physiological solution or BEB. One drop of vegetative or spore suspension containing 1 billion anthrax microbes per milliliter was added to test tube. All test tubes were placed in a thermostat at 37°. The dilutions were examined after 1-2 days, and the test tubes and particular dilutions in which growth of anthrax microbes occurred were noted.

The fourth method: Spores or culture fluid containing a particular actinomycete strain were introduced into sterile or unsterilized soddy-podzolic soil initially infected by anthrax microbe spores. For this purpose air-dried soddy-podzolic soil was moistened with sterile tap water prior to the experiments, and the soil's moisture content was increased to 60 percent of total moisture, with a consideration for the moisture introduced upon infection of the soil by anthrax microbes or actinomycete strains. During the experiments the moisture content of soil in cans was maintained within 60 percent of total moisture capacity. Samples of both experimental and control soil weighing 1 gm or 10 gm were placed in cans of different capacities, but in such a way that the thickness of the soil layer was 3-5 cm. Ten million anthrax microbe spores were added to the 1 gm soil samples and thoroughly mixed.

Actinomycete strains were introduced into the soil in different quantities:

culture fluid--from 0.1 to 0.2 ml per square centimeter of soil surface;

actinomycete spore washings from fish-meal agar or Chapek's agar, at doses from 100,000 to 1 million spores per milliliter, for every square centimeter of soil surface area.

Soil samples weighing 1 gm each were taken from 10 different places in the cans in order to determine presence of *Bac. anthracis* and determine its virulence. These samples were taken on the day the experiment was started and after 1, 2, 6, and 10 days. The samples were diluted and seeded on BEA in bacteriological dishes, and they were injected into white mice to determine their virulence.

Different methods were used to check 101 actinomycete strains, most of which were studied by only the first three methods; the rest were excluded from subsequent testing if they were inactive in relation to anthrax microbes.

The highest actinomycete activity against anthrax microbes was established for strain No 719 in dilution 6561, and for strain No 77 in the same dilution. These strains produced a clarification zone of 15 mm and more on agar.

It was also established that in terms of the testing methods described above, soddy-podzolic soil is not a suitable medium for adaptation of the tested groups of actinomycetes antagonistic to anthrax microbes.

Antibiotics tested by the first three methods and having a lethal action upon anthrax microbes--globisporin, remazin, synthomycin, gramicidin, gricemin, penicillin, biomycin, streptomycin-aureomycin, and levomycetin--failed to cause 100 percent decontamination of a superficial layer of soddy-podzolic soil containing anthrax microbes, in the doses and conditions mentioned above. Work in this direction should be continued in an effort to find ways and methods of adding, to this soil, some sort of medium which would encourage adaptation of the cited actinomycetes antagonistic to anthrax microbes.

It should be noted in conclusion that by performing extensive experiments on the use of particular plants or microbial antagonists, we can alter soil ecosystems and rehabilitate the soil of animal burial pits and other foci containing anthrax microbes.

BIBLIOGRAPHY

1. Arkhinov, V. V. "Sbornik nauchnykh trudov L'vovskogo vetzooinstituta" [Collection of Scientific Works of the L'vov Zooveterinary Institute], Vol 3, 1950, p 137.

2. Vladimirov, A. A., ARKHIV BIOL. NAUK, Vol 43, No 2-3, 1936, p 331.

3. Voznyakovskaya, Yu. M., MIKROBIOLOGIYA, No 6, 1948, p 18.

4. Grigoryan, T. M., TRUDY TURKMENSKOGO SEL'SKOKHOZYAYSTVENNOGO INSTITUTA, Vol 8, 1956, p 63.

5. Isakova, A. A., TRUDY INSTITUTA FIZIOLOGII RASTENIY IM. K. A. TIMIRYAZEVA, Vol 3, No 1, 1940, p 18.

6. Kosarev, V. A., VETERINARIYA, No 9, 1956, p 66.

7. Krasil'nikov, N. A., "Antagonizm mikrobov i antibioticheskiye veshchestva" [Antagonism Between Microbes and Antibiotics], Moscow, 1958.

8. Mirotvorskiy, K. A., IZVESTIYA TURKMENSKOGO FILIALA AN SSSR, No 3-4, 1946, p 138.

9. Presnov, I. N., TRUDY VNIIVS, No 28, 1967, p 137.

10. Pokshishevskiy, N. I., and Golovin, A. D., PRAKTICHESKAYA VETERINARIYA, No 7, 1931, p 20.

11. Tsion, R. A., and Mikhura, I. G., PRAKTICHESKAYA VETERINARIYA, No 5-6, 1930, p 426.

12. Shushayev, B. Kh., TRUDY VNIIVS, No 16, 1969.
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